

The effect of a novel electrical stimulation method for improving lower limb blood flow in healthy volunteers

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The Effect of a Novel Electrical Stimulation Method for Improving Lower Limb Blood Flow in Healthy Volunteers

By

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**This Thesis is submitted for the degree of Doctor
of Philosophy of the University of London**

Clinical Pharmacology



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September 2012

To My Parents

Statement of Originality

This is entirely my own work and all the quotations, illustrations and source materials have been appropriately acknowledged.

Huda Jawad, September 2012

Abstract

Venous Thromboembolism (VTE) is a significant preventable complication that causes morbidity and mortality not only in hospitalised patients, but also in healthy individuals. Pharmacological and mechanical prophylactic methods are available. Pharmacological methods, although are effective in reducing the incidence of VTE, the bleeding risk associated with their use is a major drawback. Besides pharmacological methods, clinical practice guidelines also recommend the use of mechanical methods (NICE, 2010b). The rationale for using mechanical methods is to increase venous return and decrease the risk of venous stasis, one of the compounding factors for VTE. As with pharmacological methods, mechanical interventions are also associated with side effects. Electrical stimulation is an alternative method, which has been shown to be effective in improving blood flow (Faghri et al., 1997, Lindstrom et al., 1982, Nicolaides et al., 1972). This method however, has not gained widespread use mainly due to the elevated discomfort associated with its use at high intensities. The limited number of available electrical stimulation devices, are complex in structure and restricts mobility. Therefore, developing an alternative technique that is effective and easy to use is justifiable. It is among our intentions in the studies presented in this thesis, to investigate the effectiveness of a novel electrical stimulation technique. The studies outlined in this thesis were carried out on healthy adult volunteers, with the intention of investigating the efficacy of a custom built neuromuscular electrical stimulation device (THRIVE) in enhancing lower limb blood flow and supporting the development of a prototype to a commercial medical device (geko™ T-1). The device activates foot and calf muscle pumps of the lower leg using OnPulse™ Technology; a software that ensures safe and controlled delivery of electrical impulses. The effect of the novel device on cardiac function was initially investigated, where the electrical stimulation device was applied bilaterally for 30 minutes at two pulse width settings 400µs and 600µs on 10 healthy subjects lying supine. A significant difference in cardiac output was reported following echocardiography assessments, $p \leq 0.05$. Similarly, a significant increase in skin microcirculatory velocity together with arterial peak velocity and blood volume flow was reported, $p \leq 0.05$. Further investigations were then performed on 10 healthy subjects seated in an airline seat for a period of 4 hours, to explore the systemic effect of the electrical stimulation device applied for a period of 5 minutes on specific blood coagulation parameters. A series of lower limb circulatory dynamic assessments were performed at baseline and at 1, 2, 3 and 4 hours. Blood coagulation parameters and blood clotting time were measured through analysis of blood withdrawn from three anatomical sites (arm, right leg and left leg). Results obtained have shown a highly significant increase in lower limb blood perfusion, $p \leq 0.001$. An enhanced fibrinolytic activity, characterised by a significant drop in tissue plasminogen antigen levels was seen. No major effects on vital signs were reported. Finally, a comparison of the effectiveness of the novel device with two leading mechanical prophylaxis devices, Huntleigh Flowtron™ Universal and Kendall SCD™ was performed on 10 healthy subjects. Each device was fitted for a period of 30 minutes followed by a 10 minute recovery phase, in a sequential manner. Colour flow Doppler ultrasound in addition to skin microcirculatory and vital

sign assessments were carried out to further assess the comparative efficacy. Results obtained demonstrate that the novel device is significantly more effective in increasing microcirculatory velocity, $p \leq 0.001$ in comparison to the IPC devices investigated. Venous blood flow measurements were also significantly higher for the geko™ T-1 device compared to the IPC devices. Furthermore, a significant increase in both arterial blood velocity and volume was reported following the use of the geko™ T-1 device.

The safety and tolerability to the device was also of prime concern. Results showed that the device was well tolerated throughout, as indicated by the minimal discomfort sensation perceived by the subjects using visual analogue scale (VAS) and verbal rating score (VRS) in all studies. In conclusion, the results obtained in this thesis indicate that the geko™ T-1 device investigated has a potential use in the prevention of deep vein thrombosis and other peripheral vascular disorders due to significant effects on circulatory dynamics in arterial, venous, and microcirculatory systems.

Publications

Abstracts

1. A.T.Tucker, DS. Bain, H.Jawad, H. Dawson, K.Adams, A. Johnston. *The development of a novel system for the prevention of venous thromboembolism*. Preventing and Managing Venous Thromboembolism (VTE). 29-30 March 2012. Melbourne, Australia.
2. H.Jawad, DS. Bain, H. Dawson, K.Adams, A. Johnston, A.T.Tucker. *OnPulse™: A potential management for venous thromboembolism*. October 2011. William Harvey Day. Barts and The London School of Medicine and Dentistry. UK.
3. H.Jawad, DS. Bain, H. Dawson, K.Adams, A. Johnston, A.T.Tucker. *A Comparative Study Investigating the Effectiveness of the geko™ Medical Device versus Intermittent Pneumatic Compression in Enhancing Lower Limb Blood Flow in Healthy Subjects*. October 2011.William Harvey Day. Barts and The London School of Medicine and Dentistry. UK.
4. H.Jawad, DS. Bain, H. Dawson, K.Adams, T. El Nahhas, A. Johnston, A.T.Tucker. *The Effect of OnPulse™ in Improving Lower Limb Blood Flow in Healthy Volunteers*. International Congress of the Union of Phlebology. European Chapter Meeting. Practical Phlebology.2011 (20); 1-72. Prague Czech Republic.
5. H.Jawad, H. Dawson, K.Adams, DS. Bain, A. Johnston, A.T.Tucker. *The Effect of a Novel Method for Improving Lower Limb Blood Flow in Healthy Volunteers*. Gulf Research Meeting. Health Care Challenges in The Gulf Region. 6-8 July 2011.Cambridge.UK.
6. H.Jawad, A.T.Tucker, A.Maass, DS. Bain, H. Dawson, M.Azzam, L.Chen, A. Johnston. *Deep Vein Thrombosis and Its Prevention via a Novel Method*. Dubai International Pharmaceuticals and Technologies Conference & Exhibition. 25-17 March 2010. Dubai. UAE.

7. A.Maass, A.T.Tucker, DS. Bain, M.Azzam, H. Dawson, H.Jawad, A. Johnston.
Electrical Stimulation of Motor Nerves. An Alternative Approach for the Enhancement of Lower Limb Blood Flow. October 2009. William Harvey Day. Barts and The London School of Medicine and Dentistry. UK

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List of Abbreviations

A2C	Apical 2 Chamber
A3C	Apical 3 Chamber
A4C	Apical 4 Chamber
AA	Arachidonic Acid
ABPI	Ankle Brachial Pressure Index
ACE	Angiotension Converting Enzyme
ACT	Automated Coagulation Timer
ADP	Adenosine Diphosphate
ASAX	Arterial Short Axis
ATP	Adenosine Triphosphate
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
CFT	Clot Formation Time
cGMP	Cyclic Guanosine Monophosphate
CT	Clotting Time
DT	Deceleration Time
DTIs	Direct Thrombin Inhibitors
DVT	Deep Vein Thrombosis
EF	Ejection Fraction
ELISA	Enzyme Linked Immunosorbent Assay
EPO	Endoperoxide
ETP	Endogenous Thrombin Potential
GCS	Graduated Compression Stockings
GSV	Greater Saphenous Vein
HRT	Hormone Replacement Therapy
IPC	Intermittent Pneumatic Compression
IPC Kendall™	Intermittent Pneumatic Compression Kendall™
IPCHF™	Intermittent Pneumatic Compression Huntleigh Flowtron™
IQR	Interquartile Range

LDF	Laser Doppler Flowmetry
LMWH	Low Molecular Weight Heparin
LSV	Lesser Saphenous Vein
LVOT VTI	Left Ventricular Outflow Tract Velocity Time Integral
LVSD	Lower Ventricular Systolic Dysfunction
MCF	Maximum Clot Formation
ML	Maximum Lysis
mL	Millilitre
NMES	Neuromuscular Electrical Stimulation
NO	Nitric Oxide
PAI	Plasminogen Activator Inhibitor
PE	Pulmonary Embolism
PGI₂	Prostaglandin I ₂
PLAX	Parasternal Long Axis
PSAX	Parasternal Short Axis
Rotem®	Rotational Thromboelastometry
SD	Standard Deviation
SPO₂	Percentage Tissue Oxygen Saturation
TAMV	Time Averaged Mean Velocity
TcPO₂	Transcutaneous Tissue Oxygen
TF	Tissue Factor
THRIVE	Thromboembolic Prevention Via Electrical Stimulation
tPA	Tissue Plasminogen Activator
TXA₂	Thromboxane A ₂
UFH	Unfractionated Heparin
VAS	Visual Analogue Scale
Vel	Velocity
VKAs	Vitamin K Antagonists
Vol Flow	Volume Flow
VRS	Verbal Rating Score
VTE	Venous Thromboembolism
vWF	Von Willebrand Factor

Chapter 1. Introduction

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE) is a major health problem that received a burst of attention in the press and clinical forums. In the year 2003, the American Public Health Association declared DVT a public health crisis. Studies from the United States have estimated the annual incidence of VTE to be approximately 150 per 100 000 of the population (Heit et al., 2001, Tsai et al., 2002). In the year 2005, the House of Commons Select Committee for Health, published a critical report stating that 25 000 patients in England die annually from VTE. The figure reported, is higher than the combined total deaths from breast cancer, AIDS, and road traffic injuries (Welch, 2010). However, unlike other diseases, VTE is largely preventable through the use of appropriate prophylactic measures in patients at higher risk. Since the year 2005, tremendous efforts and resources have been devoted to tackle this issue. Specialised teams have been established in the majority of hospitals throughout the UK, and risk assessments of VTE upon hospital admission have become mandatory. The National Institute of Clinical Excellence also addressed this problem by establishing guideline CG-92 in 2010 for the management of VTE. The guideline states assessment of patients upon hospital admission to identify those at increased risk for developing VTE and selecting the appropriate pharmacological and/or mechanical prophylaxis measures as required during their hospital stay and until recovery (NICE, 2010b). Indeed financial penalties for poor compliance have been enforced by the Department of Health, via the Commissioning for Quality and Innovation (CQUIN) target system (NICE, 2010b).

Prevention of VTE is therefore possible, through the use of the appropriate prophylaxis. Prophylaxis measures are broadly categorised as mechanical and pharmacological prophylaxis. Both strategies are effective in reducing the risk of VTE significantly compared to no prophylaxis (NICE, 2006). However, unlike pharmacological prophylaxis, mechanical methods are not associated with bleeding complications or other contraindications. They have enjoyed wide popularity, as they can be used as adjunct to pharmacological methods or as an alternative, for those patients with a particularly high

risk of bleeding. Commonly used mechanical methods include graduated compression stockings and intermittent pneumatic compression devices, which although effective they are unsatisfactory for a number of reasons. Electrical stimulation is an alternative prophylaxis method that is used during operative procedures under general anaesthesia and has been shown to effectively activate the calf muscle pump, thereby preventing DVT (Lindstrom et al., 1982, NICE, 2006, Nicolaides et al., 1972). However, electrical stimulation devices used earlier were associated with a number of shortcomings that are mainly associated with the selection of intensity currents. Whereby, the use of a low intensity current that stimulates muscles with a low level of contraction may not be sufficiently effective in promoting circulation to reduce DVT. On the contrary, the use of higher intensity currents that will induce higher levels of contraction will cause the muscles to contract sufficiently resulting in involuntary movements of the limb, which might be undesirable in certain clinical settings and exquisitely painful as well.

Objectives of the Thesis

After considering the short comings associated with the current prophylaxis measures, it is among the intention of our research, to provide a novel alternative prophylaxis method for the prevention of DVT, and or other circulatory disorders in the lower limb. A series of clinical trials were designed, conducted and presented in this thesis, with the aim of investigating the effectiveness of a novel electrical stimulation device in improving lower limb blood flow and supporting the development of the system. Increasing lower limb blood flow improves venous return and thereby reduces venous stasis. Since stasis is one of the compounding factors in the development of DVT, the risk of developing thrombosis should theoretically be reduced.

The proposed technique stimulates the motor nerves innervating the lower limb muscles at low intensity settings to promote blood circulation. The electrical stimulation is applied to the lateral popliteal nerve, in the region of the popliteal fossa that initiates isometric contraction of both the posterior and anterior lower limb muscle groups. This will result in the activation of venous muscle pump of the lower leg, in contrast to previous studies conducted, where activation of muscle pump was achieved by direct stimulation of the

calf through placing electrodes over the muscles themselves (Faghri et al., 1997, Lindstrom et al., 1982, Nicolaides et al., 1972). Clinical trials carried out to evaluate this novel technique are described in detail subsequently. The thesis is structured as follows:

Chapter 2: Background

In this chapter the circulatory system will be described and the anatomy and physiology of important blood vessels in the lower limb will be covered. The importance of haemostasis in the regulation of blood flow and in the prevention of coagulation will also be discussed. Following this general background, the concept of Venous Thromboembolism will be presented, outlining the factors leading to its development and evaluating methods of prevention.

Chapter 3: Description of the novel device investigated

This chapter includes a description of the stages of the development of the novel electrical stimulation device. The chapter will also include an overview of the devices used in the studies presented and how they were developed.

Chapter 4: Cardiac Study

The cardiac study focuses on investigating the effect of the novel electrical stimulation device initially termed THRIVE device, at two different pulse width settings in enhancing lower limb blood flow in healthy subjects lying supine. The study was also designed to evaluate the effect of using the novel electrical stimulation device on specific cardiac parameters.

Chapter 5: THRIVE Study

THRIVE study is the second clinical trial conducted with the intention of evaluating the haematological effects of the novel device (THRIVE), by investigating specific coagulation factors during the period of prolonged sitting. Assessment of the effectiveness of the novel device in enhancing lower limb blood flow at the vascular and

micro vascular level was also performed. Furthermore, the tolerance and acceptance of the subjects to the device under study were evaluated.

Chapter 6: geko™ versus IPC Comparison Study

The prototype of the electrical stimulation device used in the previous studies had gained CE marking at this stage and is now termed geko™. The intention of the study presented in this chapter, was to evaluate the performance of the novel device (geko™) in relation to commonly used mechanical prophylaxis devices in hospital settings. Therefore in this chapter, the effectiveness of the geko™ device in enhancing lower limb blood flow, will be compared to two commonly used intermittent pneumatic compression devices. Non-invasive assessments of blood flow at the vascular and micro vascular level was performed to healthy subjects lying supine. The tolerance and acceptance of the subjects to the devices tested was also evaluated.

Chapter 7: Discussion & Conclusion

In this chapter the results presented in the studies described earlier was analysed with reference to findings of previous research using similar techniques. The discussion focuses on evaluating the effects of the novel electrical stimulation from five perspectives: 1) Cardiac performance, 2) Tissue perfusion, 3) Blood coagulation, 4) Safety, and 5) Tolerance and Acceptance.

Chapter 8: Future Options

This chapter will describe briefly the potential studies that will be performed using the novel device investigated earlier.

Chapter 2. Background

2.1. The Circulatory System

The circulatory system consists of two loops that begins and ends at the heart. In addition to the heart, the circulatory system is composed of a vast array of blood vessels. The blood vessels form a tube-like network allowing the blood to flow from the heart to the tissues and back to the heart again. There are three major types of blood vessels: arteries, capillaries and veins. Arteries and veins share the same general structure. They have walls consisting of three concentric layers (tunica). The inner most and thinnest layer is the *tunica intima*, which consists of endothelial cells that is in direct contact with the blood flow. The *tunica intima* restricts the entry of substances into the vessel wall, control blood vessel diameter and regulate coagulation. In addition to endothelial cells, the *tunica intima* also consist of a sub-endothelial layer, composed mostly of connective and an internal elastic lamina that separates it from the *tunica media* layer. (Concepts, 2012, Standring et al., 2008). Tunica media is the thickest layer and is composed of muscle tissues, elastic fibres as well as collagen. The *tunica externa* is the outermost layer and consists of connective tissues, nerves and vessel capillaries especially found in large blood vessels (Standring et al., 2008). Arteries have thick walls to help withstand high pressures. The lumen part of the arteries decreases in size moving down the arterial tree. Veins on the other hand are smaller than arteries. They have a bigger lumen and thinner walls to facilitate the flow of blood under a lower pressure in comparison to that of the arteries. In comparison to the structure of arteries and veins, capillaries are smaller blood vessels with a simpler structure. They consist of a layer of squamous epithelium, though some capillaries have connective tissues as well. Capillaries have a very small lumen, which allows the exchange of nutrients between blood and tissues, refer to **Figure 1**.

Blood circulation starts at the arteries, which carry blood away from the heart. Arteries are further categorised into: pulmonary arteries and systemic arteries. Pulmonary arteries are responsible for carrying deoxygenated blood from the heart to the lungs, where it gets

oxygenated. Likewise, systemic arteries deliver oxygenated blood to the rest of the body. The main, largest systemic artery is the aorta, which originates from the heart and branches into smaller arteries. The aorta is the first blood vessel to carry blood as it leaves the heart before it begins its circulatory journey throughout the body (Weber, 2006). The aorta then branches out into smaller arteries that supply blood to the head, the heart itself and to lower regions in the body.

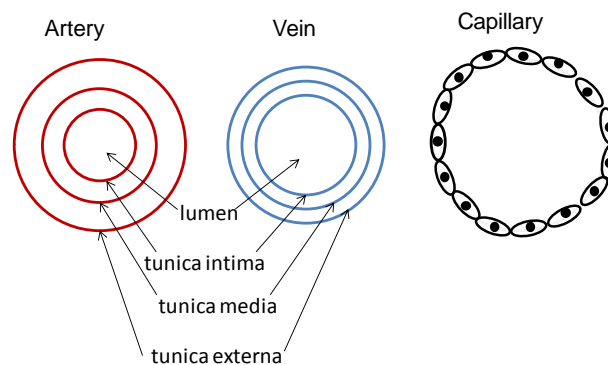


Figure 1: Structural differences between the blood vessels. Arteries and veins have walls consisting of three concentric layers. Veins are smaller than arteries with a bigger lumen and thinner walls. Capillaries are smaller blood vessels consisting of a layer of squamous epithelium with a very small lumen.

Arteries then branch to further smaller arteries termed arterioles, which regulate the flow of blood into various tissues. As it emerges from the heart, the aorta has a diameter of approximately 3cm. The diameter then decreases along the arterial tree reaching a diameter of 0.5cm in the arteries, which then further decreases into a diameter of 10 μ m in the arterioles. The smaller diameter of the arterioles provides a significant resistance to the flow of blood that creates a pressure gradient in the circulatory system. Hence, maintaining adequate blood flow to the entire body. Following blood flow in the arterioles, blood reaches the capillaries.

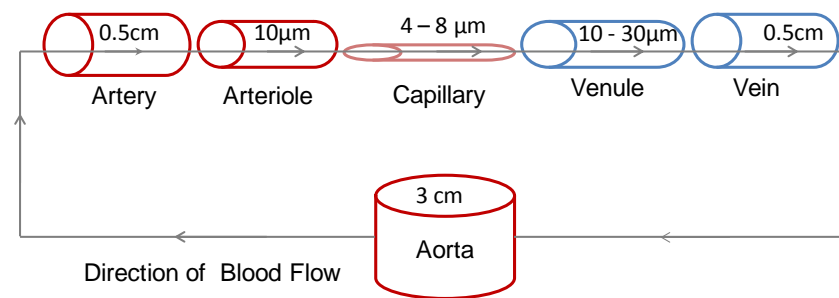


Figure 2: Sequence of Blood Flow. Blood circulation starts at the aorta followed by arteries and arterioles. It then reaches the capillaries, which drain into venules followed by the veins to be taken back to the heart.

Capillaries connect arterioles to venules, they are approximately 4-8 μm in diameter, and are composed of thin walls of endothelium. They are divided into two types, continuous and fenestrated capillaries. Continuous capillaries are responsible for the diffusion of water, small solutes and lipid soluble materials into the surrounding interstitial fluid, but prevent the loss of blood cells and plasma proteins. Unlike continuous capillaries, fenestrated capillaries contain pores across the endothelial lining that facilitates rapid exchange of water and solutes between plasma and interstitial fluid. The flow of blood through the capillaries is regulated by structures composed of muscle fibres termed precapillary sphincters that open and close allowing blood to flow when necessary. Blood received from the capillaries drain into venules in order to be transported back to the heart (**Figure 2**). Venules are usually 10-30 μm in diameter that branch into larger veins, which are approximately 0.5 cm in diameter. Veins eventually carry blood to the largest vein in the body, the vena cava that have a lumen diameter of 3 cm (Schneider et al., 2003). Veins have thin walls in comparison to arteries of similar size. However, the wall thickness of a vein is not correlated exactly to the size of the vein, and usually varies in different regions. For instance, the walls of leg veins are thicker than veins of similar size in the arm. In summary the circulation process in the body begins in the aorta, then the arteries which drain into arterioles. Blood then reaches the capillaries that further drain into venules, which in turn drain into veins and finally reach the vena cava.

As large blood vessels branch into smaller vessels, their total cross sectional area, the space through which blood flows, increases. In consequence, the blood pressure and velocity drops (**Figure 3**). The peak of pressures in the arteries is the systolic blood

pressure; the minimum is the diastolic pressure. As the blood begins its circulatory journey, the blood is driven through the arterial system by the heart. However, as the blood reaches the venous system, it is no longer moved by the heart. Instead it is the force of the muscle pumps surrounding the veins that pushes the blood, through the veins and back to the heart.

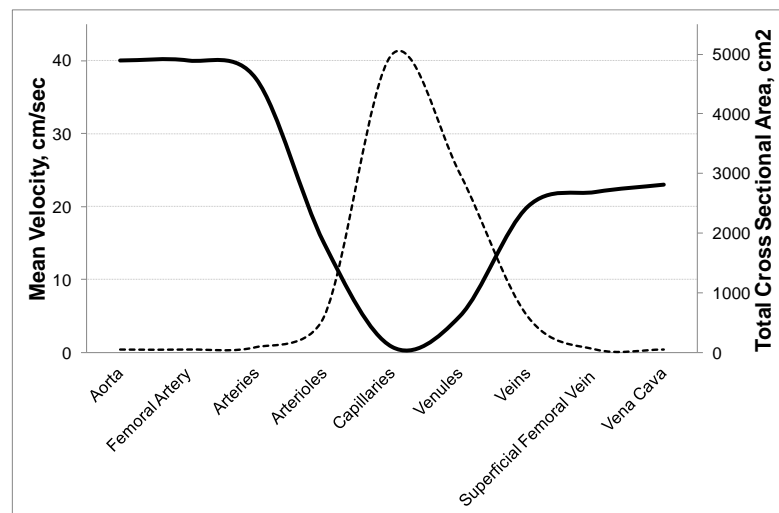


Figure 3: **Blood Flow versus Total Cross Sectional Area** adapted from (Shephard and Vanhoutte, 1980). The relationship between blood flow velocity (solid line) and total cross-sectional area (dotted line) of blood vessels is inverse. The greater the cross-sectional area, the slower the blood flows and vice versa.

2.2. Anatomy of the Venous System

The peripheral venous system acts both as a reservoir to hold excessive blood and as a conduit to return blood from the capillary network to the heart down a pressure gradient. A large part of the blood flow volume, passes into the peripheral venous system of the lower extremities, which in turn consists of three major types of veins: deep veins, superficial veins and perforating veins.

2.2.1. The Deep Veins

The deep veins of the lower extremity are the most important part of the venous system, as they are the major venous return circuit for the circulatory system. The entire venous blood eventually passes through the deep veins on its way back to the right atrium of the

heart. The deep veins of the lower extremity are divided into several segments and have the same names as their adjacent artery, refer to **Figure 4**. Starting at the ankle and moving upwards, the major deep veins are called the *tibial veins* that further divide into three pairs. *Tibial veins* then converge together forming a single vein called the *popliteal vein*, which stretches to the top of the knee joint. The *popliteal vein* then passes through the thigh, where it is known as the *femoral vein*. The *femoral vein* then reaches the area of the hip joint and becomes known as the *common femoral vein*. After passing the hip joint, the *common femoral vein* then becomes the *external iliac vein* and then the *common iliac vein*, which is located in the area surrounding the waistline (Fronek and Bergan, 2007, Ombrellaro, 2006).

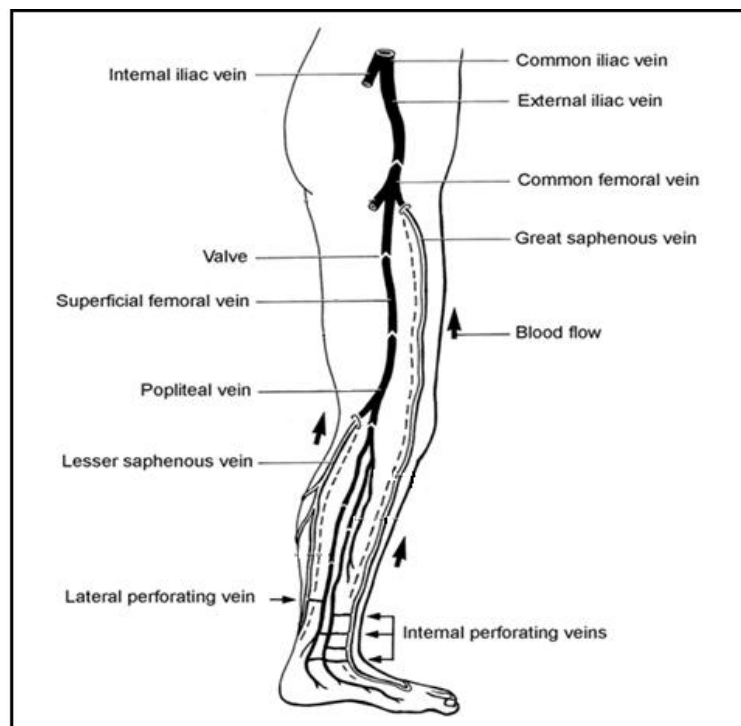


Figure 4: Venous System of the Lower Extremity. With permission of (Maggisano and Harrison, 2004). The major deep veins include the tibial veins that converge forming the popliteal vein, which stretches to the top of the knee joint. Femoral vein from the thigh reaches the area of the hip joint and becomes known as the common femoral vein. The common femoral vein then becomes the external iliac vein and then the common iliac vein, located in the waistline area.

2.2.2. The Superficial Veins

As the name implies, the superficial veins are located superficially in the subcutaneous adipose layer. These veins include the visible veins that lie right underneath the skin. The superficial veins are located in the fatty tissue layer between the muscle and the skin. Two major superficial veins in the leg are known: the Lesser Saphenous Vein (LSV), which usually runs from the ankle to the knee and the Great Saphenous Vein (GSV) that originates on the dorsum of the foot and ascends through the thigh to the groin. In the groin, the GSV then joins the common femoral vein of the deep venous system. Similarly, the LSV (**Figure 4**) originates laterally from the dorsal venous arch of the foot and travels subcutaneously behind the lateral malleolus at the ankle. It then crosses the back of the leg reaching the knee, then joins the deep venous system through connecting to the popliteal vein (Fronek and Bergan, 2007, Ombrellaro, 2006).

2.2.3. The Perforating Veins

Perforating veins are branches that penetrate the muscle in several places. These veins cross the aponeurosis linking the superficial with the deep veins. This linkage is essential for a normal venous outflow from the leg. A confusing aspect to the perforating veins is their flow direction. Some perforating veins produce a normal flow, from the superficial to the deep circulation. Others on the other hand, conduct an abnormal outflow from the deep circulation to the superficial circulation. This action is termed perforating vein reflux. Thus, any perforator vein may demonstrate a bidirectional blood flow. In the leg, there are several perforators distributed at the ankle, calf, knee, and groin area. Perforator veins include, Cockett's, Boyd's, Dodd's and Hunterian perforators (**Figure 5**) (Bergan, 2007, Brown and Pease, 2009).

2.3. Venous Pressure

Unlike arterial pressure, which is generated by muscle contractions of the heart, pressure in the venous system is influenced by gravitational forces. The effect of gravitational force is apparent when a person changes from supine to standing position. When a person is lying down, the head, heart and feet are all at the same level. The hydrostatic pressure

is nearly the same for all parts of the body, and the pressure within the blood vessels depends on the force generated by the heart.

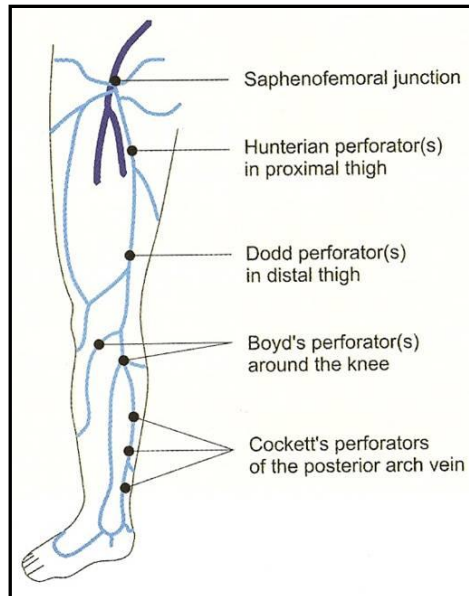


Figure 5: Perforating Veins of the Lower Leg. With permission of (Brown and Pease, 2009). Cockett's are located at the ankle/calf connecting the great saphenous vein to posterior tibial. Boyd's perforators located around the knee connects GSV to the popliteal vein. Dodd's and Hunterian perforators located at the distal and proximal thigh respectively connects the great saphenous vein to the femoral vein.

However, large hydrostatic changes occur when a person is standing. The only point in which the pressure remains constant is the hydrostatic indifferent point (zero reference pressure level). All pressure down this point is increased by 1 mmHg for each 13.6 mm distance below/ or decreased above the heart. Therefore, in an adult who is standing, the venous pressure in the feet is approximately +90 mmHg and -30 mmHg in the head. (Schneider et al., 2003). The gravitational force helps pull blood downward to the lower extremities. The force of gravity will also make it more difficult for the blood to flow upward to return the blood to the heart. However, this is made possible through the action of the muscle pump system.

2.4. The Muscle Pump System

Peripheral veins, especially those found in the legs and arms, contain one way valves, that are delicate but strong and consisting of two small leafs. The valve, when competent, stops backflow and therefore directs the blood flow towards the heart. The venous return process takes place through the regulated contraction and relaxation of muscles surrounding the veins, which in turn alternately opens and closes proximal and distal valves. For instance considering the muscle pumping action in the leg, upon muscle contraction, the deep veins of the legs are compressed causing the proximal valve leaflets to open propelling blood to the central circulation; the distal valves close obstructing the flow into the vein (**Figure 6**). During muscle relaxation, the superficial leg veins drain into the deep veins. Upon relaxation, the distal valves open allowing the blood to flow and fill the venous segment. Initially during this process, the proximal valve closes during the refill process, but then opens as the volume and pressure increases in the vein (Klabunde, 2011). Therefore, it is through this compression and relaxation cycle, that venous return is achieved. Hence, failure in the pumping cycle as a result of weakness in the muscles, incompetent valves, or thrombus formation in the blood vessels, leads to an insufficient venous return.

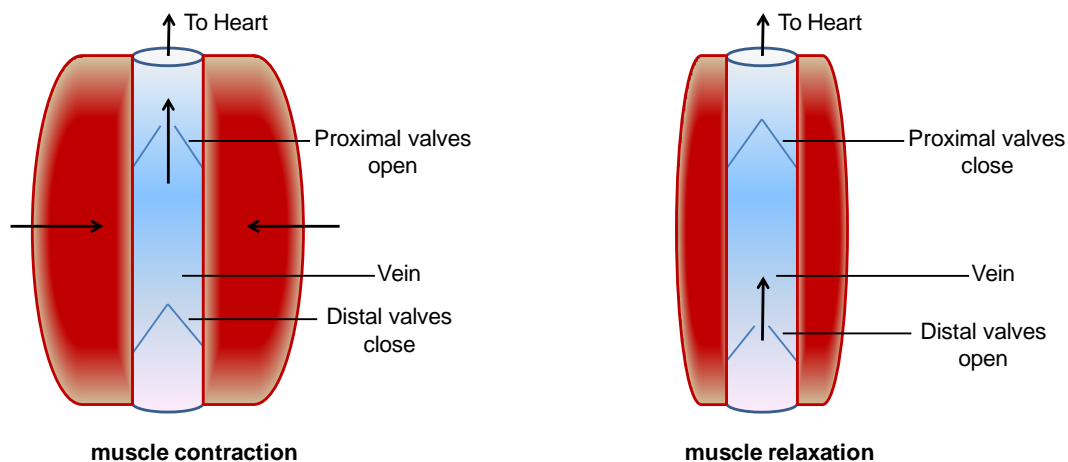


Figure 6: Muscle Pump Contraction and Relaxation adapted from (Klabunde, 2010). Muscle contraction causes the distal valve leaflets to open propelling blood to the central circulation and the proximal valves closes obstructing the flow into the vein Upon relaxation the distal valves closes during the refill process, and the proximal valves opens allowing the blood to flow and fill the venous segment.

2.5. Haemostasis

Haemostasis is a complex process involving a series of biochemical reactions to prevent excessive blood loss in the body. It is achieved through the interaction of three major constituents: clotting factors, endothelium and platelets. Each constituent in the system interacts and influences the other constituents. Under normal circumstances, haemostatic balance is maintained through the efficient interaction of all constituents in favour of anticoagulation status. Haemostasis can be broken down into two phases: *primary phase* involving the aggregation of platelets to the vessel wall and *secondary phase* that involves the formation of fibrin through a cascade of reactions including clotting factors (coagulation pathway).

2.5.1. Primary Haemostasis

The intact endothelium reduces the incidence of clotting by providing a physical barrier while secreting a series of biochemical mediators that inhibit clot formation. These mediators include vaso-relaxants such as Nitric Oxide (NO) and prostaglandin I₂ (PGI₂) commonly referred to as prostacyclin and vaso-constrictors such as Endothelin. The release of NO and vaso-constrictors from endothelial cells contributes to the relaxation of the underlying vascular smooth muscle, thereby dilating the blood vessel. PGI₂ is a prostaglandin derived from arachidonic acid (AA) through an endoperoxide (EPO) intermediate (Pettigrew, 2001). Prostaglandin I₂ and nitric oxide increase intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in platelets, which inhibit platelet activation, adhesion to the endothelium as well as aggregation. Adenosine, which is synthesised from adenosine triphosphate (ATP) and adenosine diphosphate (ADP) by endothelial cells, also increases intracellular cAMP levels in platelets (**Figure 7**).

Following endothelium disruption, primary haemostasis begins. The initial phase of haemostasis results in the formation of a primary platelet plug and encompasses the reactions of platelets, the endothelium together with a plasma protein. Endothelial disruption, caused by an injury to the blood vessel exposes the collagen found in the

subendothelium. The injury causes temporary vaso-constriction, of which not only prevents blood loss, but also slows the blood flow enhancing the adherence of platelets to the exposed sub-endothelium surfaces and to other platelets. The binding of platelets to the subendothelium, depends on the presence of collagen in the vessel wall, glycoprotein membrane receptors embedded in the surface of platelets, together with a plasma protein called von Willebrand Factor (vWF) (Welch, 2010).

Once platelets bind to the vessel wall, they become activated. Activation of platelets causes them to change their shape, activate the collagen receptors on their surface, synthesise and release a number of compounds. The compounds released by activated platelets include Endoperoxide, Thromboxane A₂ (TXA₂) together with ADP. The endoperoxide derived from arachidonic acid in platelets is converted to the prostaglandin TXA₂. Both TXA₂ formed and ADP act as platelet agonists binding to receptors on the surfaces of other platelets, causing them to activate and promote aggregation (**Figure 7**) (Pettigrew, 2001).

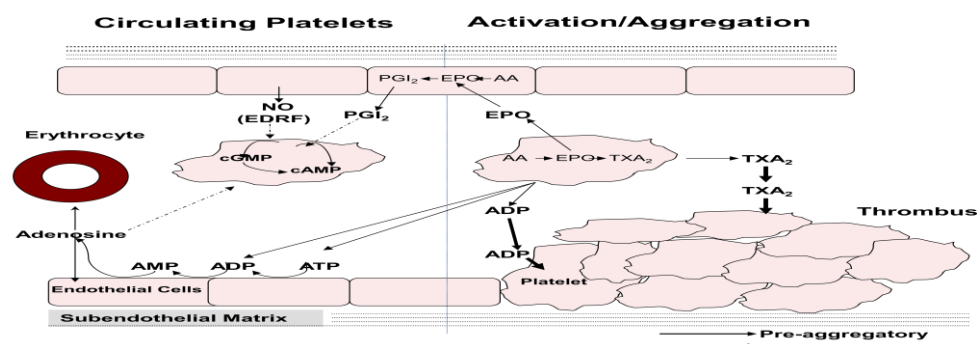


Figure 7: Platelet Antithrombotic and Prothrombotic action adapted from (Pettigrew, 2001). Nitric oxide (NO) and prostacyclin (PGI₂) synthesised from endothelial cells increase intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in platelets, which inhibit platelet activation, adhesion to the endothelium, and aggregation. Adenosine, which is synthesised from adenosine triphosphate (ATP) and adenosine diphosphate (ADP) by endothelial cells, increases intracellular cAMP levels in platelets. Activated platelets synthesise and release a number of compounds, including Endoperoxide (EPO), Thromboxane A₂ (TXA₂), and ADP. Both TXA₂ and ADP bind to receptors on the surfaces of other activate and promote platelet aggregation.

Following platelet aggregation a platelet plug is formed, however, this plug is temporary and is not strong enough. The plug will break apart and will be washed away by red blood cells, unless it becomes strengthened. This is made possible through the formation of an insoluble fibrin net during the secondary haemostasis phase.

2.5.2. Secondary Haemostasis (Coagulation Pathway)

Secondary haemostasis involves a complex coagulation cascade that ultimately results in the conversion of a soluble clot to an insoluble fibrin clot. The coagulation cascade is further divided into two pathways: intrinsic and extrinsic. Each pathway is initiated by a distinct mechanism and both converge together leading to fibrin clot formation.

2.5.2.1. Intrinsic Pathway

The intrinsic pathway also known as ‘contact activation pathway’, is initiated by the blood coming into contact with exposed collagen in the blood vessel i.e. elements that lie within the blood itself that cause damage to the blood vessel wall. Following damage to the vessel wall, a series of clotting factors are activated. The end result of this cascade is the activation of Factor X, which is an enzyme responsible for converting pro-thrombin to thrombin, the key initiator of clot formation. Thrombin converts fibrinogen to fibrin, which form a loose meshwork stabilising the clot (**Figure 8**).

2.5.2.2. Extrinsic Pathway

The extrinsic pathway also known as Tissue Factor (TF) pathway is triggered by tissue damage outside of the blood vessel, usually as a result of trauma or mechanical injury. Damage to the tissues stimulates the activation of tissue factor released by damaged tissue cells. Tissue factor is a membrane bound glycoprotein and is the primary initiator of blood coagulation. Following the activation of TF, a series of clotting factor activation takes place, eventually leading to the formation of Factor X. At this point, both the intrinsic and extrinsic pathway converges and the subsequent steps are similar. Formation of Factor X promotes the conversion of prothrombin to thrombin. Thrombin is the key step in clot formation as it mediates the transformation of fibrinogen to fibrin clot (**Figure 8**).

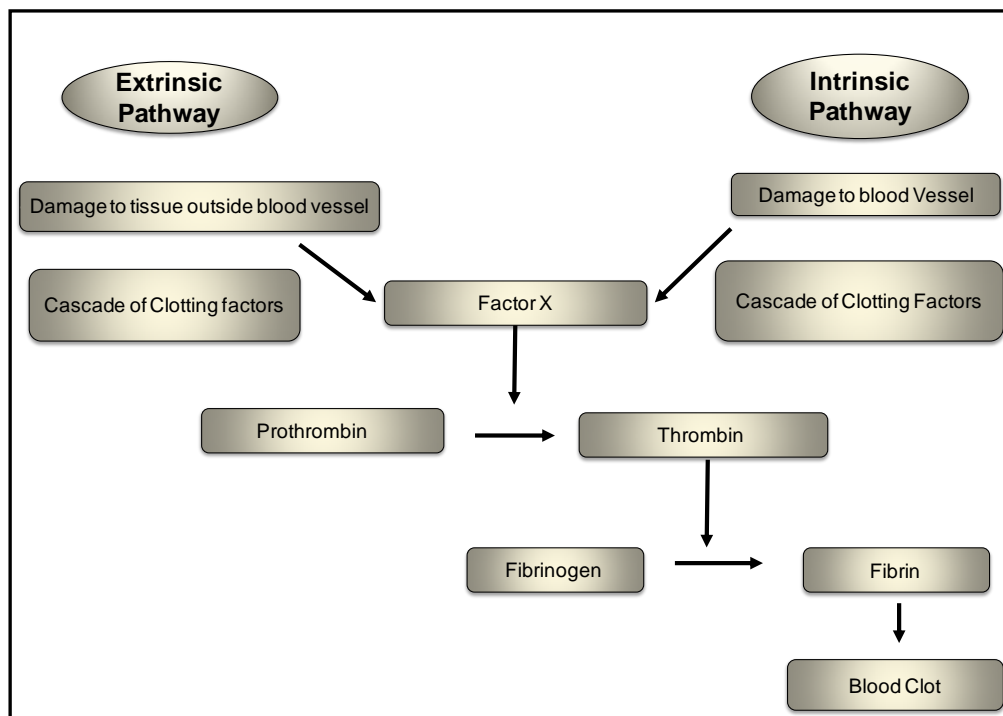


Figure 8: Coagulation Cascade. The coagulation cascade has two pathways, the intrinsic pathway; triggered by damage to the blood vessels and the extrinsic pathway that is triggered by damage to the tissue outside blood vessel. Both pathways eventually lead to the formation of a blood clot.

Following tissue repair via fibrin clot formation, the clot must then be dissolved in order for normal blood flow to resume. The dissolution of the clot occurs through a process called fibrinolysis. Fibrinolysis is the body's natural mechanism that is responsible for the degradation and removal of fibrin clots, hence restoring obstructed circulation. Plasminogen, a precursor molecule of plasmin that is produced by the liver, plays a key role in the fibrinolysis process (Chromogenix, 1995). The plasma concentration of plasminogen is about 100 – 150 mg/mL and the half-life in the circulation is about 2.8 days (Collen et al., 1972). The primary activator of plasminogen is tissue plasminogen activator (tPA), a product of endothelial cells, which activates plasminogen and converts it to plasmin. Plasma concentrations of tPA measured as tPA antigen is about 5-10 µg/L and varies greatly under different physiological conditions (Nordenhem and Wiman, 1998). In the plasma, tPA occurs mainly as a complex together with its principle plasminogen activator inhibitor, PAI-1 (Peterson et al., 2000). Plasminogen activator inhibitor is the most important inhibitor of tPA. Under normal physiological conditions,

the concentration of PAI-1 in the plasma is about 10µg/L (Urden et al., 1972). A decreased fibrinolytic activity, is mainly due to increased levels of PAI-1 (Wiman and Hamsten, 1991). Furthermore, elevated plasma concentrations of tPA antigen, which is usually found as a bound complex of tPA/PAI-1 also correlates with a decreased fibrinolytic activity. Therefore, there is a strong positive correlation between plasma PAI-1 levels and tPA antigen (Nordenhem and Wiman, 1998).

Following injury, active tPA is released from vascular endothelial cells, it then cleaves plasminogen to plasmin, which then digests the fibrin (**Figure 9**). The net result of this process is soluble degradation products to which neither plasminogen nor plasmin can bind to in addition to D-Dimer. After the release of plasminogen and plasmin, they are rapidly inactivated by their respective inhibitors.

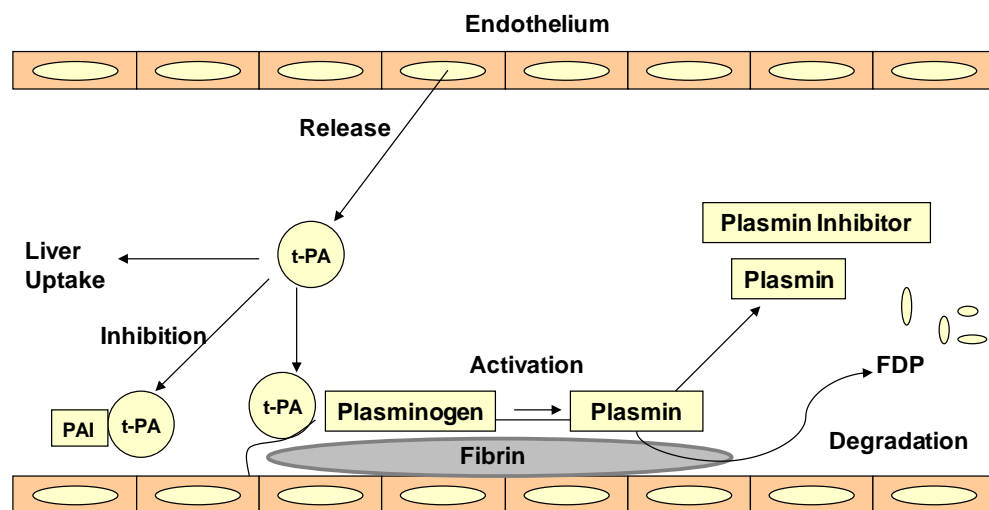


Figure 9: Schematic Representation of Fibrinolysis. Adapted from (Chromogenix, 1995). Tissue plasminogen activator (t-PA) is produced by vascular endothelial cells and is released into the circulation following stimulation. Inhibition of this process can occur through plasminogen activator inhibitor (PAI) or through plasmin inhibitor. Free t-PA together with complexed t-PA/PAI is cleared from the circulation by the liver.

D-dimer is the final degradation product formed as a result of the action of plasmin on cross-linked fibrin. It consists of two identical sub-units derived from two fibrin molecules. In an efficient fibrinolytic activity and in the presence of increased fibrin formation, an increased level of D-dimer will be present in the plasma. Hence, D-dimer

levels are increased by any condition in which fibrin is formed and degraded by plasmin (Hager and Platt, 1995).

2.6.Coagulation Assessment

It is clear from the above overview, that a range of factors are involved in the coagulation process. Therefore, assessing specific coagulation factors can provide an indication of abnormality in the coagulation process. Coagulation assessment is performed by quantitatively measuring different factors at different stages of the coagulation pathway. For the purpose of simplification, the coagulation cascade will be divided into four stages: 1. Platelet aggregation stage, 2. Thrombin formation, 3. Fibrin formation and 4. Fibrinolysis.

2.6.1. Platelet Aggregation Stage

Assessing platelet aggregation stage involves measuring prostacyclin and von Willebrand factor (vWF). As mentioned earlier, prostaglandin (PGI_2) is an inhibitor of platelet aggregation and a promoter of vasodilation. However, a key drawback of PGI_2 is its short half life; since its activity disappears within 10 minutes of yielding a stable product (6-keto $\text{PGF1}\alpha$). Therefore, it is possible to use a surrogate concentration of PGI_2 by measuring the concentration of its stable product 6-keto $\text{PGF1}\alpha$. This provides an idea of the platelet aggregatory level in a blood vessel, where decreased levels of prostaglandin have been found to be associated with vascular dysfunction and an indicator of pulmonary hypertension (Christman et al., 1992).

The second marker of platelet aggregation is von Willebrand factor, which as mentioned earlier is secreted from endothelial cells into the plasma and into the sub-endothelial cell matrix. Once released, vWF appears to play a major role in mediating platelet aggregation at the site of injury and also binds to and stabilises Factor VII that is essential for the formation of fibrin. Raised levels of vWF have been proposed as a possible indicator of endothelial dysfunction, which predisposes thrombosis (Badimon et al., 1992, Blann, 1993, Boneu et al., 1975). Raised vWF levels have also been associated with an increase in thromboembolic events, as supported by Koster et al., who found that

vWF levels increased in patients with a history of deep vein thrombosis (Koster et al., 1995).

2.6.2. Thrombin Formation

Thrombin formation is a result of complex enzymatic mechanisms taking place. Therefore, assessment of thrombin formation is useful in the clinical diagnosis of venous thromboembolism and is made possible through measuring the Endogenous Thrombin Potential (ETP). ETP indicates the thrombin generation capacity of the plasma, which is considered to be one of the main determinants of haemostasis and thrombosis (Hemker and Beguin, 1995). Substances that have the ability to reduce the thrombin generation capacity in plasma such as anticoagulants have an antithrombotic and haemorrhagic effect. On the contrary, all conditions that increase thrombin generation such as deficiencies of anti-thrombin result in thrombosis.

2.6.3. Fibrin Formation

As mentioned earlier, following the formation of thrombin, fibrin is formed from its precursor fibrinogen. With the advancement of technology, it is now possible to get a quick measurement of the clotting time (fibrin formation), through the use of an Automated Coagulation Timer technology (ACT). Using a photo optical system, ACT detects the clot formation of a freshly withdrawn blood sample and displays the results in seconds.

Additionally, it is also possible to get a detailed analysis of the dynamics of fibrin formation, stabilisation, as well as fibrin dissolution through the use of a Rotational Thromboelastometry (ROTEM®) technique. In comparison to ACT, ROTEM® is more advanced in the sense that it can measure both the intrinsic and extrinsic coagulation pathways, providing detailed information about the journey of a clot. The key parameters measured using ROTEM® include, Clotting Time (CT), Clot Formation Time (CFT), Maximum Clot Firmness (MCF) and Maximum Clot Lysis (ML).

2.6.4. Fibrinolysis

In addition to using the ROTEM® technique to measure the clot lysis time, it is also possible to get an indication of the clot lysis (fibrinolysis) by measuring the concentration of specific markers. These include Tissue Plasminogen Activator (tPA) and D-dimer levels. As mentioned earlier, tPA is the primary initiator of fibrinolysis. An adequate amount of tPA is essential for maintaining the endothelium in a non-thrombogenic state. For instance, a deficiency in tPA will result in a reduced capacity to degrade fibrin, resulting in thrombosis. Furthermore, D-dimer, the final degradation product of fibrin can be measured, providing a quick indication of abnormal clotting. An elevated level of D-dimer is an indication of the presence of abnormally high levels of cross-linked fibrin degradation products. This implies that a significant clot formation and clot breakdown have been taking place in the body.

2.7. Venous Thromboembolism

Venous thromboembolism (VTE) has evoked great interest over the past few years in many fields; such as the media, airline industry and public health institutions. Venous thrombosis is characterised by the formation of a blood clot (thrombus) in a vein, which if left untreated may cause partial or complete obstruction to blood flow. VTE incorporates Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). DVT is the term used to describe the formation of a blood clot in the deep veins. If the blood clot is left untreated, it may break into fragments called emboli, and travel to the lungs forming PE. Although clots can form in the upper extremities, the most common site for thrombus formation is the deep veins of the calves, thigh and pelvis (Welch, 2010).

2.7.1. Epidemiology of VTE

The precise incidence of VTE is unknown, as most studies are limited by the inaccuracy of clinical diagnosis. Epidemiological studies have been carried out worldwide, with the majority of the reports from the United States. Based on these reports, the annual incidence of VTE in the United States was found to range between 70 to 150 per 100 000 persons (Anderson et al., 1991, Heit et al., 2001, Silverstein et al., 1998, Tsai et al.,

2002). In Europe, data from studies carried out in France and Sweden reported an occurrence rate of VTE 160 to 180 per 100 000 persons (Cohen et al., 2007). Although such data has contributed greatly to our understanding of the epidemiology of VTE, the total burden of this disease on the health care system remains underestimated. This is mainly due to the absence of data on the undiagnosed or misdiagnosed VTE cases together with the unrecognised VTE related deaths. Therefore, in an attempt to describe the health burden of VTE within the European Union (EU), a study carried out by the VTE Impact Assessment group in Europe (VITAE) investigated the annual number of non fatal incidents and recurrent VTE events, as well as VTE related deaths in six countries within the EU. The VITAE study estimated that the total number of non fatal VTE events is almost 460 000 for DVT and 300 000 cases for PE out of approximately 760 000 events. The total VTE related deaths is estimated 370 000 out of which 26 000 are treated and 215 000 are not treated (Cohen et al., 2007). Hence, based on those figures it is in no doubt that VTE is an important public health issue that requires greater attention in an attempt to reduce the health burden of this disease.

2.7.2. Risk Factors of VTE

The underlying aetiology for the development of VTE was described by ‘Virchow’s Triad’, outlined by Rudolph Virchow in 1856, who proposed that three main factors are associated with thrombosis: 1. venous stasis 2. damage to the vein wall and 3. blood hyper-coagulability. Thus, it is believed that any risk factor that induces VTE can be characterised by the existence of one or more of the components of Virchow’s Triad.

The presence of risk factors plays an important role in assessing the probability of developing VTE. Moreover, risk factor assessment permits successful thromboprophylaxis and prevent further development of complications associated with VTE. Hence, reduce the health burden of the disease. A summary of some of the risk factors predisposing to thrombosis is found in the sections below. It is essential to recognise that the predictive values of these risk factors are not equal. Prior to providing prophylaxis, physicians should consider both the strength of individual risk factors and the cumulative weight of all risk factors. A range of risk factors with their corresponding

odds ratios are listed in **Table 1**. Strong risk factors that have an odds ratio of greater than 10 justify prophylaxis against VTE.

<i>Strong Risk Factors (Odds Ratio >10)</i>
Hip fracture
Hip or knee arthroplasty
Major general surgery
Major general trauma
Spinal cord surgery
<i>Moderate Risk Factors (Odds Ratio 2-9)</i>
Chemotherapy
Congestive heart or respiratory failure
Hormone replacement therapy
Malignancy
Oral contraceptive therapy
Stroke
Pregnancy/postpartum
Previous venous thromboembolism
<i>Weak Risk Factors (Odds Ratio < 2)</i>
Bed rest > 3 days
Immobility due to sitting (prolonged travel)
Advanced age
Laparoscopic surgery
Obesity
Pregnancy/antepartum
Varicose veins

Table 1: Risk Factors for VTE. (Anderson and Spencer, 2003)

2.7.2.1. Advance Age

Venous thromboembolism is a major health problem, mainly affecting the elderly population. The risk of VTE is quite low before the age of 40. For instance, in persons 15 years old or younger, there is less than 5 cases per 100 000 of the population annually (Anderson et al., 1991, White et al., 2003). However, it increases dramatically after the age of 75. As found by Kniffin et al., the annual rate of DVT in the United States increases from 180 per 100 000 persons at the age of 65 to 69 years to 310 by the age of 85 to 99 years (Kniffin et al., 1994). The increased risk with advanced age is mainly due to the presence of additional factors and / or complications that might lead to changes in the venous system and hence, result in thrombosis.

2.7.2.2. Prolonged Travel

There has been much debate in the media regarding the development of VTE following long hours of travel. Deep vein thrombosis have been incorrectly termed ‘Economy Class Syndrome’, as some of the DVT cases have been reported in airline passengers who travelled in economy class. However, DVT is not restricted to airline traveller nor to travellers on economy class. It can affect people using any other forms of travel including cars, trains or any vehicle. A number of studies have been carried out to determine the actual incidence of DVT following long haul flights (Belcaro et al., 2001, Ferrari et al., 1999, O’Keeffe and Baglin, 2003). For instance when investigating the incidence of VTE within 24 hours of travelling for long hours in the LONFIT 1 study, it was found that the incidence was 0% (0/355) in patients without any risk factors for VTE. In comparison, in higher risk patients 2.8% developed DVT following long hours of travel (Belcaro et al., 2001). High risk patients were defined as those with a pre-history for DVT, have limited mobility due to bone or joint problems, have severe obesity and exhibit a coagulation disorder. Furthermore, in a randomised trial, it was reported that 10% of passengers undertaking long flights (>8 hours) developed asymptomatic calf DVT detected with duplex ultrasound (Scurr et al., 2001).

A number of factors associated with air travel are thought to play a contributory role in the development of DVT. During periods of quiet sitting a number of rheological and biochemical changes have been reported to occur in the limb. Prolonged sitting in a cramped position with limited space results in restriction of the natural venous pumps. In the sitting position blood flow velocity is reduced by two-thirds compared with lying in the supine position (Delis et al., 2004, Wright and Osborn, 1952). Cramped position may also cause venous stasis and further induce endothelial damage by compression of the popliteal vein at the edge of the seat.

2.7.2.3. Ethnicity

Racial and ethnic differences have been shown to influence incidence rates of VTE. In a study carried out in California, it was found that the annual incidence of DVT was 23 per 100 000 in Caucasians, 29.3 in African Americans, 13.9 in Hispanics, and 6 in Asians

(Bulger et al., 2004). Similarly, studies of hospitalised patients in North America and Hong Kong have also shown that Asian patients, including those of Chinese origin have a significantly lower incidence rates than people of African origin. The reason behind such differences has not been found, but it has been suggested that it could be due to certain genetic traits that increase the risk of developing VTE. As reported by Joynt et al., 2009, Chinese people tend to have a low level of Factor V Leiden gene mutation, which is a factor responsible for increased coagulation (Joynt et al., 2009). Furthermore, it has been found that Asian people tend to have lower mean fibrinogen and a decreased hyper coagulation Factor VIIc and VIIIc concentrations, which are associated with an increased risk to DVT if present in high concentrations (Ho et al., 2000, Ho et al., 1995).

2.7.2.4. Pregnancy & Oestrogen Therapy

Women face several conditions in their lifetime that increase their risk of developing VTE. These conditions include pregnancy, use of oral contraceptives as well as hormone replacement therapy (HRT). In comparison to non pregnant women, VTE have been shown to increase during pregnancy by 4 to 5 fold (Heit et al., 2005, James, 2009). Pregnancy is associated with hyper-coagulation as well as venous stasis, which occur by the end of the first trimester. Pregnancy is also associated with endothelial damage to pelvic vessels, which usually takes place during vaginal or abdominal delivery. Hence, as all initiating factors for thrombosis are present, this sets the scene for thrombosis to take place.

Further epidemiological studies have clearly established an association between the use of oral contraceptives and VTE (Bulger et al., 2004). The risk has been found to be dependent on the oestrogen dose, with preparations containing greater than 50µg of oestrogen being associated with the highest risk. Thus, although the doses of oestrogen in oral contraceptive formulations have now been reduced to 30µg, the reduction in oestrogen dose has not reduced the thrombotic risk. Several studies have shown that oral contraceptives with a reduced dose of oestrogen (< 50µg) are associated with a 3 to 6 fold increase in relative risk of VTE (World Health Organization, 1995). The risk is highest during the first year of using contraceptives and is heightened with prolonged use (Herings et al., 1999).

Similarly as with oral contraceptives, the use of hormone replacement therapy has been found to increase the risk of VTE. The increased risk is also associated with the dose of oestrogen present in the hormonal replacement therapy, which is one-fifth the dose found in the oral contraceptives. However, published data has shown a 2 to 4 fold increase in relative risk of VTE in current users of HRT as compared with non-users, with a heightened risk observed during the first year of use (Bloemenkamp et al., 1995).

2.7.2.5. Obesity

Obesity is defined as excessive accumulation of fat and is described by a body mass index (BMI) equal to or above 30 kg/m² (World Health Organization, 2006). Although it has not consistently been identified as an independent risk factor for VTE, obesity is an important modifiable risk factor. Several studies have reported an increased risk of DVT within the obese populations. For example, in an epidemiological study including 106 patients, (Samama, 2000) reported a 2 fold increase in risk of DVT occurrence among patients with a BMI greater than 30kg/m².

The patho-physiological correlation between thrombosis and BMI has been extensively studied. There are a number of studies substantiating a positive correlation between BMI and several coagulation factors such as Factor VII, Factor IX and D-dimer levels (De Lorenzo et al., 1998, Serrano, 1998, Shipfinger et al., 1998). Furthermore, it has been established that obese people tend to have a further increased thrombotic risk when exposed to additional risk factors such as oral contraceptives. For instance, in a single study examining the correlation between obesity and oral contraceptive use, a 1.8 to 3 fold increased risk of DVT was found among oral contraceptive users (Nightingale et al., 2000). This was confirmed by Abdollahi et al., who reported a 10 fold increased risk of DVT among obese women using oral contraceptives as compared to women of normal weight who did not use oral contraceptives (Abdollahi et al., 2003).

It is clear from the above figures, that the presence of various risk factors influence the development of VTE. It has been shown that the greater the number of risk factors, an individual holds, the greater the likelihood for developing VTE. For example, in a community review carried out on 1231 consecutive patients treated for VTE, 96% were

found to have at least one risk factor and the risk increases with the number of risk factors an individual holds (Anderson and Spencer, 2003, Anderson et al., 1991).

2.7.2.6. Surgery

As a result of an accumulation of risk factors, hospitalised patients have more than a 100 fold increased risk of VTE (Heit et al., 2001). Hospitalised patients might be subjected to prolonged immobility, surgical procedures, or paralysis as a result of anaesthesia. Such factors will induce changes in all three components of the Virchow's Triad. Surgical procedures result in vessel wall damage and hyper coagulability. Similarly, prolonged immobility deprives the veins of the legs of activity resulting in venous stasis (Welch, 2010). The type of surgical procedures performed determines the risk of VTE, as certain procedures increase the risk of VTE, refer to **Table 2**. Patients having spinal cord surgery have the highest risk (60-80%) for developing DVT, compared to 10-20% for general medical patients. It is therefore necessary to carry out a pre-assessment of the VTE risks, bleeding risks, and the type of thromboprophylaxis that can be provided.

<i>Condition</i>	<i>DVT Prevalence (%)</i>
General Medical Patients	10-20
General Surgery	15-40
Major Gynaecological Surgery	15-40
Major Urological Surgery	15-40
Neurosurgery	15-40
Stroke	20-40
Congestive Heart Failure	20-40
Hip/Knee arthroplasty,	40-60
Major Trauma	40-80
Spinal Cord Surgery	60-80

Table 2: Risk of DVT in hospitalised patients in the absence of thromboprophylaxis. (Geerts et al., 2008, Spyropoulos, 2005).

2.8. VTE Prophylaxis

VTE is a major health problem, however unlike other diseases; it is preventable through the use of appropriate thromboprophylaxis. The House of Commons Health Committee in the year 2005 reported that an estimated 25 000 people in the UK die from preventable hospital acquired VTE each year (House of Commons Health Committee, 2005). The

reported number includes patients admitted to the hospital for medical care as well as surgery. The reason behind such a high incidence has been attributed to the inconsistent use of thromboprophylactic measures in hospital settings. Therefore, to tackle this problem the National Institute for Health and Clinical Excellence (NICE) issued Guideline CG92 in the year 2010 (NICE, 2010b). The guideline provides recommendations for assessing and reducing the risk of VTE in patients in hospitalised settings. It also outlines the most cost effective prophylaxis methods that may be used for VTE prophylaxis taking into account the risks associated with the various prophylaxis measures used (NICE, 2010b). Current preventative measures available fall into two categories: a) pharmacological and b) mechanical methods.

2.8.1. Pharmacological Prophylaxis

Pharmacological prophylaxis involves the use of medicinal agents in the management of VTE. A range of antithrombotic agents are available, and are classified based on their mechanism of action. The most widely used agents are anticoagulants, which block the production and activation of clotting factors. Three classes of anticoagulants are available, vitamin K antagonists, heparins and direct thrombin inhibitors. Depending on the patient's condition, these drugs are usually given prior to, during, or following surgical procedures.

2.8.1.1. Vitamin K Antagonists (VKAs)

Vitamin K antagonists (VKAs) are commonly referred to as 'oral anticoagulants', examples include *Warfarin* and *Coumarin* that have been available for more than 50 years. VKAs are blood thinning agents that have no direct effect on clot synthesis; therefore they do not break clots. The aim of using VKAs is to prevent further clot progression (Baker et al., 2004). VKAs act by lowering the amount of active vitamin K available for the activation of certain clotting factors. However, a major side effect to VKAs is bleeding and the need for constant monitoring. Hence, in certain conditions, it might be more beneficial to replace the use of VKAs by other agents.

2.8.1.2. Heparins

Heparin is an anticoagulant that has been discovered to exhibit antithrombotic properties over 90 years ago. It has been extensively used as a treatment for VTE for the past 50 years. Heparin prolongs bleeding time and increases vessel wall permeability (Hirsh et al., 2001, Hirsh and Raschke, 2004). Three types of heparins are in use: i) Unfractionated Heparin (UFH), ii) Low Molecular Weight Heparin (LMWH), iii) Synthetic Pentasaccharides (Factor Xa inhibitors)

i) Unfractionated Heparin (UFH)

Unfractionated heparin is a naturally occurring glycosaminoglycan derived from either pig intestine or bovine lung tissue, with a molecular weight ranging between 5 000 to 35 000 daltons (Scottish Intercollegiate Guidelines Network, 2011). UFH acts by inactivating the activation of clotting factors, mainly thrombin and Factor Xa. This results in the inhibition of fibrin formation. UFH is administered parenterally either through intravenous infusion that has an immediate effect (30 min), or a subcutaneous injection with a delayed (2 hrs), but a more prolonged effect (10 hrs) (Scottish Intercollegiate Guidelines Network, 2011). Although UFH is an effective form of antithrombotic treatment, however, as with *Warfarin* it is associated with a bleeding risk in addition to other complications. Therefore, another type of heparin (LMWH) may be used instead.

ii) Low Molecular Weight Heparin (LMWH)

Low molecular weight heparin is largely taking over as the agent of choice for both prevention and treatment of VTE. It is synthesised from UFH through a depolymerisation reaction resulting in the production of smaller molecules ranging between 4000 to 6000 daltons in size (Raskob et al., 2001). LMWH is equally effective as UFH, although it is more expensive. However, its long term use allows outpatient management and reduces hospital stay, which makes it more economical in the long run (Hirsh et al., 2001). Examples of commercially available LMWH include: *Ardeparin*, *Dalteparin*, *Enoxaparin Sodium*, *Rivaparin* and *Tinzaparin*. The effectiveness of LMWH in reducing

the risk of VTE has been investigated through a meta- analysis performed by Mismetti et al in the year 2000 that showed that both UFH and LMWH are equally effective at reducing the incidence of VTE, but LMWH is safer with a 52% lower bleeding risk (Mismetti et al., 2000). The mechanism of action of LMWH is similar to that of UFH however; LMWH has the advantage of having a greater bioavailability following subcutaneous injection. Hence, LMWHs are more effective as antithrombotic agents when given a once or twice daily dose in comparison to a twice or three times daily dosage for UFH.

iii) Synthetic Penta - saccharides (Factor Xa inhibitors)

Despite the proven efficacy of LMWH, synthetic penta - saccharides have been shown to be more effective than LMWH in reducing asymptomatic form of DVT (Bounameaux and Perneger, 2002). An example of a synthetic penta-saccharide is Fondaparinux, which contains the same penta-saccharide sequence found in UFH and LMWH. Fondaparinux acts as a catalyst, enhancing the inhibition of Factor Xa, which in turn inhibits thrombin formation (Hyers, 2003). It is rapidly absorbed following subcutaneous administration and has a long half life of approximately 17 hours, allowing a once daily dosing with a rapid onset of action (Hyers, 2003). Evaluation of the side effects have shown that in addition to the risk of bleeding Fondaparinux holds, it may also result in thrombocytopenia (Papadopoulos et al., 2007).

Another antithrombotic agent that inhibits Factor Xa is *Danaparoid*, a low molecular weight heparin. It is mainly used in practice as an alternative treatment in patients who develop heparin-induced thrombocytopenia (HIT) from heparin therapy. *Danaparoid* is also safe for use during pregnancy, as it does not cross the placenta. However, a major disadvantage is its prolonged half life that ranges from 17 to 28 hours.

2.8.1.3. Direct Thrombin Inhibitors (DTIs)

Direct thrombin inhibitors are agents that are capable of inhibiting both circulating thrombin and clot-bound thrombin. As *Danaparoid*, DTIs are also used an alternative treatment in patients who develop heparin induced thrombocytopenia. Despite this

advantage, the major drawback of DTIs is their bleeding risk (Hyers, 2003). The first DTIs to be used medically was *Hirudin*; a 65 amino acid polypeptide. Several other DTIs were then developed including *Bivalirudin*, *Dabigatran* as well as, *Ximelagatran* (Raskob et al., 2001).

2.8.2. Mechanical Prophylaxis

Although mechanical prophylaxis methods are not as well researched as pharmacological prophylaxis, they have gained wide popularity especially since they are not associated with bleeding risk. Mechanical prophylaxis have therefore been primarily recommended as an alternative thromboprophylaxis method in patients subject to high bleeding risk, or as an adjunct to pharmacological prophylaxis (Geerts et al., 2008). As mentioned earlier, any factor inducing VTE can be characterised by the presence of one or more of the components of the Virchow's triad. Therefore, any thromboprophylaxis method used produces their effect by targeting components of the Virchow's triad; altering venous stasis, vessel wall damage, and/or hyper coagulability. Mechanical methods are further divided into two groups: compression methods and electrical stimulation.

2.8.2.1. Compression Methods

2.8.2.1.1. Graduated Compression Stockings (GCS)

Generally accepted compression methods include Graduated Compression Stockings (GCS) and Intermittent Pneumatic Compression (IPC) devices. Graduated Compression Stockings are the most commonly used method of mechanical compression. They are available as full length extending up to the mid thigh, or below the knee. GCS exert their action, through applying a graduated pressure on the lower leg, with the pressure being the highest at the ankle. GCS compresses the leg, reducing the cross sectional area of the veins, which in turn reduces venous stasis by increasing blood flow velocity. The graduated pressure together with muscle activity in the lower limb, results in the movement of blood from the superficial venous system to the deep venous system reducing venous stasis and preventing clot formation, thereby potentially preventing DVT (Amaragiti and Lees, 2000).

In a systematic review, a 52% reduction in the relative risk of DVT was estimated following the use of graduated compression stockings as compared to no prophylaxis (Amaragiti and Lees, 2000). In addition to its moderate effectiveness in preventing DVT, graduated compression stockings offer the advantage of being used during recumbency, as well as during immobility. However, to exert their beneficial action, the correct measurements of the leg must be taken. As with other prophylactic measures, the use of GCS is also associated with some adverse effects. They are immensely uncomfortable to wear and difficult to apply, this often results in poor patient compliance. Compression stockings also give rise to a hygiene problem as they have to be washed regularly and replaced at least within six months of first usage. Frequent washing and wearing causes a loss of elasticity resulting in loss of adequate compression. Adverse effects such as hypersensitivity reactions are also possible. For example, in a multicentre controlled trial examining the effectiveness of thigh length graduated compression stockings in reducing DVT following stroke in 2518 patients, adverse effects such as skin breaks, ulcers, blisters, and skin necrosis were significantly more common in patients using compression stockings. In addition to the adverse effect, results from the CLOTS study, investigating the effectiveness of thigh length GCS, were discouraging, as GCS was not clinically effective in reducing the risk of DVT in patients with stroke (CLOTS, 2009).

2.8.2.1.2. Intermittent Pneumatic Compression (IPC)

Intermittent Pneumatic Compression (IPC) is another form of mechanical compression prophylaxis. IPC devices operate by squeezing blood from the underlying deep veins, which if the valves are competent will be displaced proximally (Morris and Woodcock, 2004). Compression is achieved through the action of a pump that inflates and deflates air bladders within cuffs that cover the whole leg, the calf or just the feet. The deflation action causes the refill of the veins, ensuring the periodic flow of the blood through the deep veins, thereby preventing venous stasis. Published data have shown that IPC action is not only targeted at preventing venous stasis, but has also been found to stimulate fibrinolytic activity (Christen et al., 1997, Comerota et al., 1997b, Labropoulos et al., 1999). IPC have been found effective in reducing the risk of DVT by 60% in post operative patients, compared to patients receiving no pharmacological or mechanical

prophylaxis (Urbankova et al., 2006). Despite their proven efficacy, IPC devices cannot be used during mobility, as they are often bulky and not portable. They can also be hot and induce sweating beneath the sleeves.

2.8.2.2. Electrical Stimulation Methods

Electrical stimulation is the third type of mechanical prophylaxis. Published data on the effectiveness of electrical stimulation dates back to the year 1972, where initial studies were performed investigating the use of electrical stimulation for the prevention of thromboembolic complications following surgery (Nicolaidis et al., 1972). As the research presented in this thesis is based on the use of electrical stimulation technology, a detailed overview of the technique will be addressed in the following chapter.

Chapter 3. The Physiology of Neuro-muscular Electrical Stimulation

The membrane of the living cell maintains different concentrations of positively and negatively charged ions, so that in effect each cell acts like a tiny battery (Mayor, 2007). The exterior of the cell is charged more positively than the interior.

Information is transmitted around the body along the neuron, the functional unit of the nervous system in the form of electrical signals. Information is transmitted across gaps, called synapses in the form of neurotransmitter chemicals (Mayor, 2007). The nervous system consist of the brain and the spinal cord, which make up the central nervous system (CNS). The rest of the nervous system is termed the peripheral nervous system. Changes in the external and external environment are sensed by receptors in the form of impulses. The nerves that carry impulses from the receptors to the CNS, are termed sensory nerves or afferents. The nerves that carry impulses from the CNS to the muscles or glands are the motor nerves, or *efferents* (Premkumar, 2004).

The neurons communicate with each other, by changing the electrical potential inside the cell. This takes place through the movement of ions in and out of the cell.

3.1. Resting Membrane Potential

Nerves exhibit a resting membrane potential, such that the nerve membrane is polarised, even in its resting state. Polarisation is achieved by the unequal movement of ions across the membrane, which uses energy to create this state. In its resting state, a potential difference of -70mV is maintained across a nerve cell refer to **Figure 10**. The concentration difference of ions as well as the difference in electrical charges, serve as a force to reinforce or oppose movement of ions hence, transmitting an action potential.

3.2. Action Membrane Potential

Action potential occurs when a nerve is stimulated, resulting in a temporary change of state of the nerve membrane, altering the pump and ion channel activity of the membrane. The inside of the cell can then either become less negative, where the state is known as the depolarisation or more negative in a state termed hyperpolarisation. Following the stimuli action, the cell returns to its original resting potential state and this is known as repolarisation, refer to **Figure 10**. If the stimulus is repeated, another action potential takes place. In some neurons however, several action potentials are produced continuously, as long as the stimulus remains and the generation of action potential stops once the stimulus is terminated. Furthermore, the strength of the stimulus generated in the body is proportional to the frequency of the action potential generated per second and not amplitude.

When considering this in relation to the use of electrical stimulation, a similar outcome is achieved (i.e. electrical stimulation generates action potential followed by repolarisation to return to the resting potential state). However, the only difference is that the action potential is ‘forced’ by a source outside the body for instance; using an electrical current or a pulse of electricity, refer to **Figure 10**.

3.3. Nerve Membrane Threshold

The threshold level at which an action potential will be produced varies from one neuron to another (Premkumar, 2004). Stimulating a nerve cell to produce an action potential, requires a stimulus that is sufficient enough to push the membrane potential over its threshold value. If a weaker stimulus is applied, the nerve threshold will not be reached. Thus, although membrane changes are taking place, no action potential will be produced. The use of a stimulus beyond the threshold level, is therefore essential to stimulate a nerve to produce an action potential. It does not matter how much stronger the stimulation is, as the stimulation strength might stimulate more nerves, but will not influence the magnitude of the action potential.

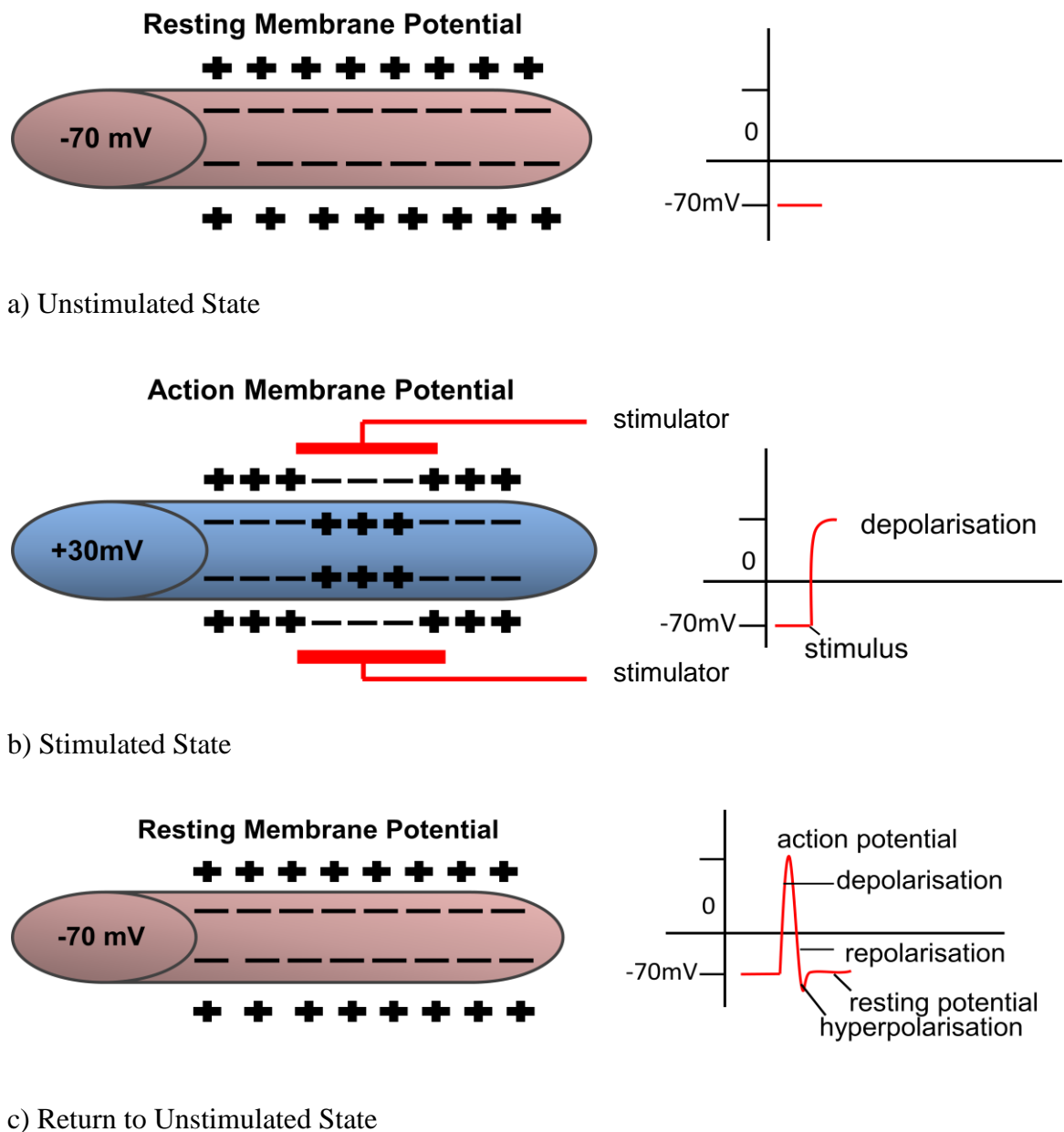


Figure 10: Nerve Membrane Potentials. Adapted from (Premkumar, 2004, Watson, 2010). a) Resting membrane potential is the unstimulated state, characterised by a potential difference of -70mV. b) Action potential takes place when the nerve is stimulated, resulting in a potential change of +30 Mv with less negative ions inside the cell. c) Following Action potential, the stimulus is removed and the nerve cell returns to its original unstimulated state.

3.4. Electrical Stimulation

Electrical stimulation is a vast area, as there appears a range of electrical stimulation devices with diverse properties. Novel electrical stimulation devices are available in the market. However, there appears a great confusion as to how each device operates, what they are best used for, and how effective they are in doing the job they are intended to do.

The research described in the subsequent chapters, focuses on the development of a novel neuromuscular electrical stimulation device. Neuromuscular electrical stimulation (NMES) is the process of evoking an action potential in a nerve through the delivery of an electric charge to the skin. Neuromuscular electrical stimulation involves stimulating motor nerves to elicit muscle contraction. Electrical stimulation is achieved through the use of two electrodes that are connected to a stimulator by wires although; recent developments of implanted electrodes are available. Electrodes usually have a sticky conductive gel coating at the bottom that enables them to adhere to the skin and ensure effective transmission of the charge from the electrode to the skin. Electrical stimulation is conducted through the use of specialised equipment that includes a stimulus generator, electrodes, power source and a user control unit. Stimulation parameters (frequency, pulse duration, amplitude and waveform) are chosen in relation to the type of nerve or muscle being stimulated. The key issue is to set the device in such a way as to stimulate the target nerves as effectively and efficiently as possible (Watson, 2010). Stimulation of sensory nerves will bring about a sensory outcome. Similarly, stimulation of motor nerves will bring about motor effects.

3.5. Applications of Electrical Stimulation

Electrical stimulation was initially used for the prevention of DVT in the year 1972 during operative procedures (Nicolaidis et al., 1972). The electrical stimulation was applied by a mains-operated Thrombophylactor (Stanley Cox Medical Equipment Division of Rank Precision Industries Ltd.), which is a device where electrodes can be connected to, consisting of a series of knobs for the selection of the desired intensity. The research performed demonstrated that calf muscle electrical stimulation using a single 50

millisecond wave current in the range of 12 to 15 pulses per minute under general anaesthesia is effective in eliminating stasis during operation, resulting in a 92% reduction in the incidence of DVT. In addition to the bulkiness of the electrical stimulator device, a key drawback in this study is that the stimulation cannot be used during postoperative periods on conscious patients, due to the high electrical stimulation intensities. Furthermore, the electrical stimulation cannot be used conveniently in patients undergoing orthopaedic surgery on their legs, as there will be issues regarding the positioning of the electrical stimulation (Nicolaidis et al., 1972).

Further evidence on the effectiveness of electrical stimulation in the prevention of DVT was documented by Lindstrom et al in 1982, who showed that stimulation of the calf muscles with groups of impulses giving short periods of tetanus, reduces peroperative venous stasis considerably more than single impulses in patients undergoing abdominal surgery (Lindstrom et al., 1982). The electrical stimulation was applied using pre-gelled electrodes, which are in turn connected to a stimulator device giving galvanic square wave electrical impulses. The current range used during the study was 40-50mA, that was applied to patients anaesthetised with barbiturate, $N_2O_2 + O_2$ and morphine, and were given muscle relaxants (Lindstrom et al., 1982). Although the study further proves the effectiveness of using electrical stimulation in reducing venous stasis, the strength of the impulse used is still high and can only be used under general anaesthesia.

The effectiveness of electrical stimulation in alleviating venous stasis was further demonstrated by (Faghri et al., 1997). Electrical stimulation to the calf and thigh muscle pumps was given to patients prior to and during total hip and knee arthroplasty under general anaesthesia. The stimulation was applied using carbonised rubber skin surface electrodes (Medtronic Inc.), which are connected through long wires to an eight-channel electrical stimulator that induced rhythmic pulsatile isometric contractions in the calf and thigh muscles. The electrical stimulation used had a pulse width of 300 μ s, a pulse current of 65mA and a pulse frequency of 35Hz (Faghri et al., 1997). Results of the study demonstrated the effectiveness of electrical stimulations in activating the skeletal muscle pumps to promote venous return as shown by the higher stroke volume and cardiac output. Thus, although the results of this study are in agreement with results of previous

studies using electrical stimulations, however, the problem of pain associated with the use of high intensities of electrical stimulations, which makes it usable only under general anaesthesia remains unsolved. Pain reduction is therefore an important issue that needs to be considered when developing an accepted method that can be used in clinical practice.

Following those earlier studies, trials investigating the effectiveness of direct electrical stimulation technology in improving blood flow continued (Kaplan et al., 2002, Lyons et al., 2002, Morita et al., 2006). Various electrical stimulation devices have been tested in a variety of ways on various population groups, with various outcome measurements. Lyons et al. (2002) demonstrated that the use of calf neuromuscular electrical stimulation at a frequency of 25 to 35Hz and a pulse width setting of 200 and 300 μ s increases the popliteal venous flow velocity. Furthermore, Kaplan et al. (2002) demonstrated the effectiveness of using a neuromuscular electrical stimulation technology over an extended period. In their study, the femoral and popliteal venous flow velocities in the stimulated leg were significantly higher than those in the non-stimulated leg throughout a period of 4 hours (Morita et al., 2006). Considering the studies mentioned above, the effectiveness of neuromuscular electrical stimulation in increasing venous blood flow, hence venous stasis is apparent. However, the intensity of the stimulation used is still high and restricts mobility as the effect of this type of stimulation is muscular tetanus. Also, data on the compliance of patients to those techniques is absent. Further research based on the use of electrical stimulation is therefore justifiable, in an attempt to develop a technology that is more effective in increasing blood flow, does not restrict mobility, is simpler in design, less painful and better tolerated.

Chapter 4. Investigating a Novel Electrical Stimulation Device

Using the preceding knowledge, an attempt was made to test and evaluate a novel technique that enhances blood flow via transcutaneous electrical nerve stimulation. The studies presented in this thesis investigated the effectiveness of a novel neuromuscular electrical stimulation technology in enhancing blood flow, which is believed to potentially prevent DVT. Initially, a custom built electrical stimulator device consisting of two geometrically corrected electrodes connected by wires to a stimulator box was developed by the Vascular Scientist Dr. Arthur Tucker and Biomedical Engineer Dr. Duncan Bain, refer to **Figure 11**. The device was termed THRIVE referring to thromboembolic prevention via electrical stimulation. The electrodes were placed over the common peroneal nerve (also called the lateral or medial popliteal nerves) located in the region of the popliteal fossa behind the knee. Stimulation of the peroneal nerve has the advantage of initiating contraction of both the posterior and anterior muscle groups. Simultaneous stimulation of such muscle groups results in a more stable isometric contraction that enhances blood flow from the lower limbs back to the heart, thus increasing venous return. A substantial increase in lower limb blood flow was demonstrated to improve venous return and thereby reduce venous stasis. As stasis is one of the compounding factors in the development of DVT, the risk of developing thrombosis should theoretically be reduced. Stimulation of the peroneal nerve also results in the contraction of the foot muscles (foot pump). This will further stimulate the emptying of the venous blood and enhance blood flow. Additional advantages of the selected stimulation of the peroneal nerve are that the resultant muscle stimulation is compatible with standing and walking.

Preliminary studies carried out using this novel technique have demonstrated the efficacy of the device in enhancing blood flow at the distal and femoral vein (Tucker et al., 2010). It has also been found that a more efficient pumping action is achieved using a series of short pulses, rather than a series of prolonged contractions. Taking this into

consideration, the stimulation sequence of the novel device was adjusted using a series of single pulses as opposed to a series of prolonged pulses.



Figure 11: A Custom Built Electrical Stimulator Device. The custom built device (THRIVE) developed by Dr Arthur Tucker and Dr Duncan Bain consists of two geometrically corrected electrodes connected by wires to a stimulator box.

In light of the promising results obtained by preliminary studies performed by (Tucker et al., 2010), further studies described in this thesis were carried out. The cardiac study, described in chapter 3 was the first study performed with the intention of further confirming the efficacy of the novel device in enhancing blood flow using different pulse width settings and evaluating the effect on specific cardiac parameters. The cardiac study was followed by the THRIVE study, described in Chapter 4, which was primarily designed to investigate the systemic effect of the electrical stimulation device on specific blood coagulation parameters. The THRIVE study also further investigated blood flow parameters to confirm previous findings obtained.

Through conducting the Cardiac and the THRIVE studies, it was found that the combined use of single pulses together with the stimulation of the peroneal nerve not only improved comfort to the user, but also greatly reduced current consumption. However, the separate circuit boxes, cables and electrode placement was not convenient or clinically appropriate. This presented the possibility of further developing the THRIVE device, into a low

power device, powered by a single-button cell, similar to the type used to power wrist watches. As clinical investigations on the THRIVE device were taking place, work was in progress on improving the overall design and specification of the device. Further developments of the device took place, to develop a compact, integral device whereby the control and induction circuit, battery and electrodes were all contained on a single self adhesive strip. Such development presented advantages in terms of convenience, disposal and infection control. The integral construction of the THRIVE device also provided the potential for a fixed geometry and orientation of the electrodes; this overcomes the inconsistency associated with orienting the positive and negative electrodes.

Following the completion of the cardiac and THRIVE study, the device was developed in collaboration with First Kind Ltd, High Wycombe UK into a complete integral, self adhesive unit comprising a battery, circuit and electrodes. The device was accredited with the widely recognised CE marking into a medical device named geko™ T-1 that is powered by OnPulse™ technology, refer to **Figure 12**. OnPulse™ is a software that ensures safe and controlled delivery of electrical impulses via the geko™ electrodes. The geko™ T-1 is a portable, compact device resembling a wrist watch. It is simple, self-adhesive, disposable, battery powered, one-size-fits all device that allows unrestricted ambulation while active. The electrical current supplied by the device is limited to 27mA and the interval is constant and is regulated to repeat the cycle of blood movement every second. The geko™ T-1 device consists of 7 pulse width settings ranging from 70µs to 560µs. The desired pulse width setting can be selected by the on/off switch button; refer to **Table 3** for more specifications of the device and Appendix 25 for the device instruction manual.



Figure 12: geko™ T-1 Electrical Stimulation Device. The (THRIVE) device was further developed by First Kind Ltd. High Wycombe UK, into geko™ T-1 device that is powered by OnPulse™ technology.

Weight	18g
Dimensions	149mm x 42mm x 11mm
Stimulation Mode	7 selectable pulse width
Pulse Width	70,100,140,200,280,400,560 μ s
Pulse Current	27 mA (\pm 15%) constant current
Repetition Rate	1 Hz (\pm 5%)

Table 3: Specifications of the geko™ Device.

After developing the CE marked geko™ device, further investigations were performed by carrying out the geko™ versus IPC comparison study, described in chapter 5. The intention of the study was to compare the effectiveness of the device in enhancing blood flow with the most commonly used mechanical prophylaxis device (intermittent pneumatic compression) used in hospitals.

The studies described in this thesis were all performed by a research team that comprised of a chief investigator, clinical investigator ultrasonographer, independent accredited echocardiographers (Cardiac study), anaesthetists (THRIVE Study) and myself (PhD Student). The tasks carried out by each team member are summarised in **Table 4**.

Team Member	Responsibility
Chief Investigator	Consenting and screening volunteers
	Blood withdrawal
	Fitting Devices (geko™, IPC, Laser Doppler
	Flowmetry, Pulse Oximetry and Transcutaneous
	Oxygen Saturation device (TcPO ₂)
Ultrasonographer	Colour Flow Duplex Ultrasound Measurements
Anaesthetist	Needle cannulation of the foot and arm
Clinical Investigator	Rotational Thromboelastometry Assessment
Echo cardiographer	Echocardiography Scans
Myself (PhD Student)	Protocol Design and Literature Review
	Laboratory Management
	Explaining Study Procedure
	Completing Case Report Forms
	Performing Automated Coagulation Timer
	Assessments
	Blood centrifugation, Laboratory Analysis using
	Enzyme Linked Immunosorbent Assay (ELISA),
	Statistical analysis of all parameters measured

Table 4: Research Team Responsibilities.

Chapter 5. The Effect of THRIVE Device on Cardiac Performance in Healthy Volunteers: A Pilot Study

5.1. Introduction

Heart failure is a clinical syndrome that occurs when the heart is unable to pump enough blood to meet the demands of the body (Petersen et al., 2002). Heart failure is the result of structural or functional abnormalities of the heart. The symptoms most commonly encountered are breathlessness, fatigue and peripheral oedema (NICE, 2010a). Heart failure can clinically present with predominantly systolic or diastolic dysfunction or both. Diastolic dysfunction is the state where the heart loses its ability to relax. The heart is therefore unable to fill with blood. Therefore, to compensate for this state, the heart sometimes pumps out higher volumes of blood than in normal situations. Systolic dysfunction on the other hand, occurs when the heart is unable to pump out sufficient blood, which results in more blood remaining in the ventricles. This state is also known as left ventricular systolic dysfunction (LVSD). There is a strong association between left ventricular systolic dysfunction and heart failure. About two-fifths of people with heart failure will have impaired left ventricular systolic function (defined as an ejection fraction of less than 40%), and around half of those with left ventricular systolic dysfunction have definite heart failure (Davies et al., 2001).

In the United Kingdom, there are about 63 000 cases of heart failure each year, of whom around 34 000 are men and 29 000 are women. Hospital statistics have shown that in the last ten years, the number of heart failure hospital admissions in the UK has increased by around 5% in men and 4% in women aged 45 years and older (Davies et al., 2001). The prevalence of heart failure increases steeply with age in both men and women. While around one in 35 people aged 65 to 74 years have heart failure, the number increases to about one in 15 of those aged 75 to 84 years, and just over one in 7 of those aged 85 and above. Considering all ages, heart failure is more common in men than women (Cowie et al., 1999, Davies et al., 2001). Mortality rates, although falling, figures are still high in

the UK. In the year 2000 just under 10 000 deaths due to heart failure were recorded (Davies et al., 2001). Furthermore, heart failure is also associated with severe morbidity. People with heart failure tend to have a poor quality of life relative to people with other chronic conditions. The main group of drugs used to treat heart failure are angiotensin converting enzyme (ACE) inhibitors, beta-adrenoceptor blocking drugs (beta-blockers) as well as diuretics. However, although these therapeutic agents have decreased the overall mortality associated with heart failure, the proportions of patients that are alive but have severe symptoms has increased.

Heart failure patients can suffer from peripheral oedema due to the inadequate pumping action of the heart. As a result of this condition, blood tends to pool into the veins and subsequently in the capillaries under the influence of gravity. High pressure in the capillaries increases the hydrostatic pressure and causes fluid accumulation into the interstitial space (Casey, 2004). In addition to the severe discomfort, oedema can further lead to chronic skin ulceration, particularly if it occurs in combination with other illnesses such as diabetes and peripheral vascular disease. This further increases the burden on the patient and may lead to hospital admission and the need for special wound care.

Reduction of peripheral oedema is possible through stimulating calf and foot muscle pumps, which will in turn decrease the venous pressure in the peripheral region and augment blood flow towards the heart. Initial experiments using an electrical nerve stimulation device (THRIVE) have been shown to increase venous return from the lower limb and significantly increase blood flow velocity at the capillary level (Tucker et al., 2010). This increase potentially decreases the capillary blood pressure and hence the forces that lead to fluid leaking from the capillaries into the interstitial space, which as mentioned earlier is one of the presumed mechanisms for oedema.

5.2. Objectives

The key objective to carrying out this study is to investigate whether the known increase in venous return from the lower limb leads to an enhancement in cardiac performance in

healthy individuals. This is achieved through carrying out a comprehensive evaluation of cardiovascular function, by measuring cardiac output, left ventricular filling and ejection fraction. This will provide a clinical justification of the efficacy and safety of the device to be applied to patients with heart failure.

5.3. Study Approval

The study sponsored by Barts and The London School of Medicine and Dentistry and funded by Sky Medical Technology Limited was approved by the National Research Ethics Service (NRES), Brent Medical Ethics Committee, reference number 05/Q0408/14 on 7th January 2009, see Appendix 1.

5.4. Materials & Methods

5.4.1. Volunteers

Nine healthy volunteers (7 males and 2 Females) were recruited by advertisement to staff and students at Barts and The London, Queen Mary University, Barts and The London NHS Trust, and to the general community as approved by the Medical Ethics Committee, refer to **Table 5**. Of the nine subjects recruited, 5 were Caucasians, 2 were Asians and 2 were from other ethnic origins. All subjects complied with the study protocol.

Parameters	Mean (SD)	Median [range]
Sex; M: F	7: 2	
Age; Years	37.33 (8.14)	33 [30-48]
Body Mass Index (BMI)	25.07 (3.77)	24.2 [20.3 – 31.2]

Table 5 : Demographic Data for Cardiac Study Volunteers.

Volunteers were instructed to have a light breakfast, avoiding fatty foods and caffeine containing products. They were also asked to abstain from vigorous exercise from the previous evening onwards. All volunteers attended the Royal London Hospital, Barts and The London NHS Trust, where assessments were performed. Upon arrival they were provided with information sheets and written informed consent was obtained prior to the study refer to Appendix 2 & 3. A screening evaluation was performed to each participant,

which included a medical history, physical examination together with vital signs (blood pressure, heart rate and respiratory rate) and colour flow duplex ultrasound of the lower legs to exclude DVT. The specific inclusion and exclusion criteria are presented in **Table 6** and **Table 7**.

Health	Good general health
Age	Between 18 – 65
Medical History	No abnormal findings, absence of DVT and haematological disorders
Body Mass Index (BMI)	Between 18 – 34
Drugs	No history of drug abuse (including alcohol) licit or illicit
Medication	No medication usage during 30 days preceding the study or during the course of the study.

Table 6: Cardiac Study Inclusion Criteria.

5.4.2. Electrical Nerve Stimulation Device

A custom built electrical stimulation device (THRIVE) was fitted bilaterally to the common peroneal nerve. For each volunteer two different pulse width settings, 400µs and 600µs were used consecutively. In both settings, the frequency used was 3Hz and the device was current modulated to provide a peak current of 20mA. The duration of each stimulation programme was 30 minutes for each setting. The stimulation device used consists of two electrodes connected to a stimulator box and placed over the common peroneal nerve, which is related to the reaction of the venous muscle pumps of the lower leg (foot and calf) refer to **Figure 13**.

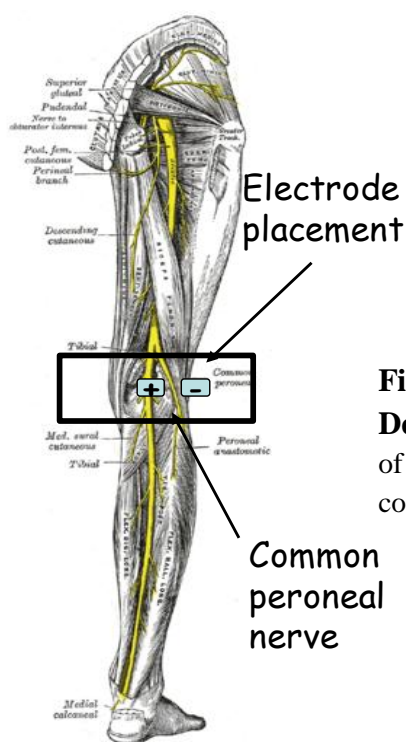


Figure 13: Positioning of Electrical Stimulation Device. A custom built electrical stimulation device consisting of a pair of electrodes attached to a box was placed over the common peroneal nerve at the back of the knee.

Health	Organ dysfunction, any clinically significant deviation from normal in the physical determinations
Age	< 18 or > 65 years
Medical History	Haematological disorders, previous DVT/PE, varicose veins or lower limb ulceration, musculoskeletal disorders, recent surgery and recent trauma to lower limb; and history of gastrointestinal, hepatic, renal, cardiovascular, endocrine, neurological, dermatological, rheumatological, metabolic (including diabetes), psychiatric, or systemic disease judged to be significant
Body Mass Index (BMI)	Chronic obesity (BMI >34 kg/m ²)
Pregnancy	Positive pregnancy test
Ankle Brachial Pressure Index (ABPI)	Peripheral arterial disease (ABPI < 0.9)
Medication	Consumption of any medication in the previous 30 days
Participation in other clinical study	Participation in any clinical study during the 8 weeks preceding the active period of the study

Table 7: Cardiac Study Exclusion Criteria.

5.5. Study Procedure

The study was carried out in a quiet, stable, draught free environment. After obtaining written consent and completing a screening evaluation, volunteers were clad in shorts, lay supine on a padded table with their head slightly tilted. Following 30 minutes of supine rest, baseline echocardiography assessments were performed by two independently accredited echocardiographers. Electrical stimulation using the THRIVE device, was then applied at a lower pulse width setting (400 μ s), followed by a higher pulse width setting (600 μ s). Each stimulation was active for a period of 30 minutes and 10 minutes resting period was used to separate each pulse width setting, to allow vascular re-equilibration. Echocardiography assessments were performed with the device still active, 5 minutes before the end of each stimulation period.

Two dimensional, motion mode, together with Tissue Doppler Imaging echocardiography were performed to obtain a comprehensive evaluation of the cardiovascular function. Two dimensional imaging was used to assess ventricular and valvular movement. Motion mode, usually referred to as M-mode was used to measure dimensions and timing of specific cardiac events. Furthermore, tissue Doppler imaging was used to assess valvular flow patterns, left ventricular diastolic function, together with cardiac output (Kaddoura, 2009). The imaging techniques were performed in six standard views; parasternal long axis (PLAX), parasternal short axis (PSAX), apical 4 chamber (A4C), apical 2 chamber (A2C), apical 3 chamber (A3C), as well as arterial short axis (ASAX). To obtain two dimensional images and for quality purpose, a single focus was used, the frame rate was set at 75/s and three cardiac cycles were stored.

Cardiovascular evaluation included testing the pumping action of the heart by measuring biplane left ventricular ejection fraction (EF), calculated using Simpson's method. Simpson's method treats the ventricles of the heart as a series of discs, where the ventricular length is divided into 20 equal sections. Calculations are then made using the length and diameters of the discs. Moreover, left ventricular filling was assessed by measuring the mitral inflow velocity as well as the left ventricular diastolic volume, which was also calculated using the Simpson's method. In addition to those parameters,

assessment of the cardiac output was made by measuring the left ventricular outflow tract velocity time interval (LVOT VTI).

Besides echocardiography, colour flow duplex ultrasound imaging (Philips IU 22; Philips Healthcare, USA) was performed on the femoral artery (15cm proximal to the patella) by an accredited vascular ultrasonographer. Parameters measured included femoral vessel diameter, peak velocity, blood volume flow together with cross sectional area. Owing to an excessive artefact caused by leg motion when the stimulation device is active, venous parameters were not measured. Both echocardiography and colour flow duplex ultrasound measurements were measured at baseline and 5 minutes before the end of the stimulation period. Measurements obtained were manually recorded in the subject's case report form at the end of each study period.

Furthermore, laser Doppler flowmetry (LDF) was used to measure microcirculatory blood flow through tissue capillaries, arterioles and venules. The laser Doppler flowmeter used in this study was (Laser Doppler Perfusion & Temperature Monitor DRT4; Moor Instruments Ltd, UK). Laser Doppler flowmetry works when a beam of laser light is directed through optical fibres to the probe, where it penetrates the skin to a depth of 1-1.5 mm and is partly reflected and scattered. Light scattered back from the tissue by moving red blood cells, then undergoes a frequency shift that is proportional to the velocity of moving objects (Flux), and is expressed in volts (De Graaff et al., 2001).). In this study, probes were attached bilaterally to the foot for each volunteer. Following zero calibration and stabilisation of LDF signals, skin blood flow was recorded for 5 minutes at baseline and at the end of the stimulation period, while the device is still active. LDF signals recorded for 5 minutes, were then processed by a computer analysis system, CED 1401 using Spike 2 software (Cambridge Electronic Design Ltd., Cambridge UK), which averages the recordings, thereby reducing the effect of artefact. Using the data obtained from the LDF analysis, adjusted LDF was calculated by deducting the zero calibration values from the actual LDF measurements obtained.

5.6. Statistical Analysis

All assessments were acquired at baseline and during the use of the electrical stimulation at different pulse widths. The baseline values obtained for each assessment were used as a reference range. Although reporting percentage change from baseline is often used to report results, this has been found to be statistically inefficient and does not correct for imbalance between groups at baseline (Vickers, 2001). For this reason the data were analysed untransformed. A comparison of the data before and after the use of the electrical stimulation device was then made. Statistical analysis was performed using Minitab 16 software (Minitab Ltd, UK). Analysis of variance using adjusted sum of squares followed by Dunnett's Test was conducted for each parameter tested. A p-value of ≤ 0.05 was considered statistically significant. The data demonstrated in the results section represents the mean of data obtained from 9 volunteers.

5.7. Results

5.7.1. Doppler Ultrasound Assessments

5.7.1.1. Arterial Volume Flow

Analysis of the data using a linear model showed that there is a highly significant difference between the different pulse widths, 400 μ s and 600 μ s as compared to the baseline, $p \leq 0.05$. The mean values of the femoral arterial volume flow, obtained following the use of the electrical nerve stimulation device were higher in comparison to the baseline values, refer to **Figure 14**. The highest mean value was obtained following the use of pulse width 600 μ s. The mean (SD) values were 174.1 mL/min (39.2) at baseline, 258.8 mL/min (65.6) following pulse width 400 μ s and 273.1 mL/min (97.1) following pulse width 600 μ s. Results obtained when calculating the average percentage change in comparison to the baseline, were approximately similar for both pulse widths. Blood volume flow increased by 55% and by 54 % following the use of pulse width 400 μ s and 600 μ s respectively. This shows that both pulse width settings were equally effective in enhancing the arterial blood volume in comparison to the baseline.

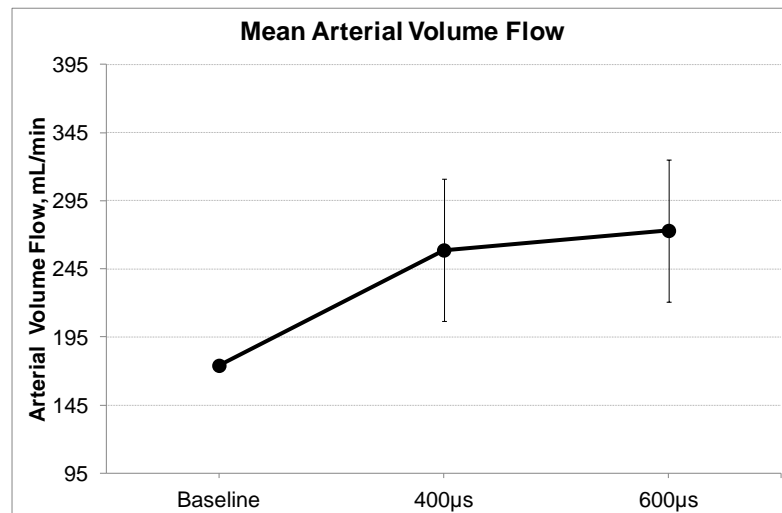


Figure 14: Cardiac Study Mean Arterial Volume Flow. Error bars represent 95% confidence interval of the difference from baseline, $p \leq 0.05$.

5.7.1.2. Peak Velocity

Measurements of arterial blood velocity obtained following analysis of variance showed a highly significant difference between the different settings as compared to the baseline, $p \leq 0.05$. Peak blood velocity measurements increased following electrical stimulation. The mean (SD) values were 81.19 cm/sec (13.62) at baseline, 101.60 cm/sec (22.43) following pulse width 400µs and 100.90 cm/sec (26.37) following pulse width 600µs. The use of electrical nerve stimulation at both pulse widths gave rise to equal increases in blood velocity by 24% as compared to the baseline, refer to **Figure 15**.

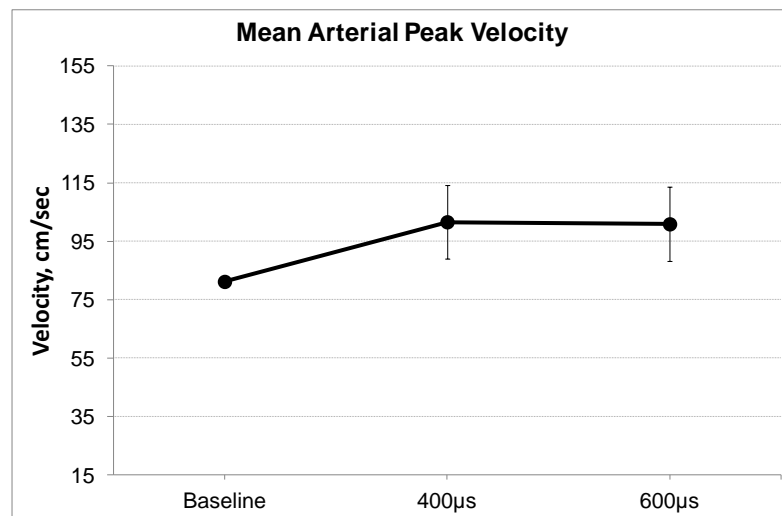


Figure 15: Cardiac Study Mean Arterial Peak Velocity. Error bars represent 95% confidence interval of the difference from baseline, $p \leq 0.05$.

5.7.1.3. Cross Sectional Area

Unlike blood volume flow and peak velocity, analysis of the change in the femoral vessel cross sectional area following the use of electrical nerve stimulation was not significant, $p > 0.05$. The mean (SD) values obtained following analysis of variance were in the same range following the electrical stimulation when compared to the baseline. The values were 0.34 cm^2 (0.10) for the baseline, 0.32 cm^2 (0.14) and 0.33 cm^2 (0.11) for pulse widths 400µs and 600µs respectively, refer to **Figure 16**.

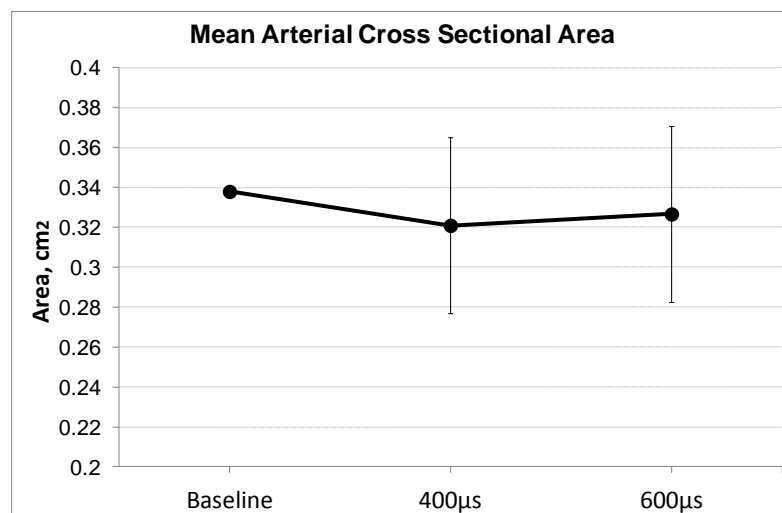


Figure 16: Cardiac Study Mean Arterial Cross Sectional Area. Error bars represent 95% confidence interval of the difference from baseline, $p > 0.05$.

5.7.1.4. Vessel Diameter

Analysis of variance for the change in femoral vessel diameter was also not significant following electrical stimulation as compared to the baseline, $p > 0.05$. The mean (SD) values were again of similar range; 0.64 mm (0.10) was obtained at baseline, 0.63 mm (0.12) following pulse width 400 μ s and 0.64 mm (0.11) following pulse width 600 μ s, refer to **Figure 17**.

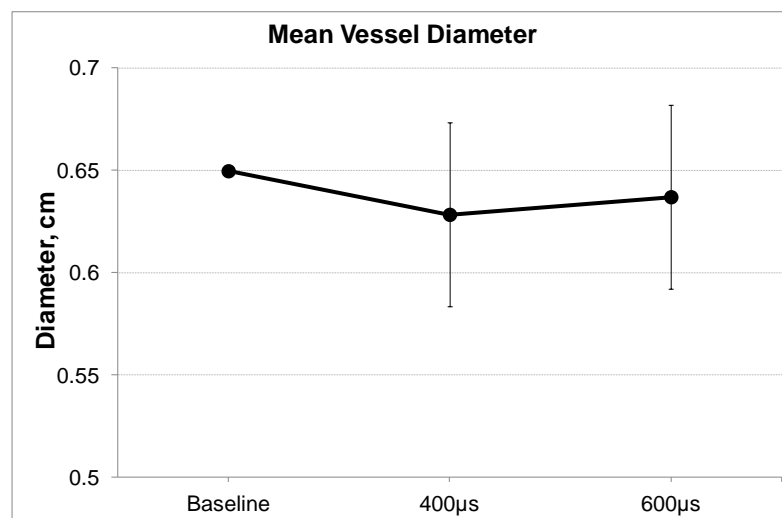


Figure 17: Cardiac Study Mean Vessel Diameter. Error bars represent 95% confidence interval of the difference from baseline, $p > 0.05$.

5.7.2. Laser Doppler Flowmetry (LDF) Assessments

A highly significant difference was reported following assessment of skin microcirculation measurements using LDF, $p \leq 0.05$. A substantial increase was seen following the use of electrical nerve stimulation in comparison to the baseline. The use of pulse width 600 μ s was associated with a higher increase in LDF measurements in comparison to the use of pulse width 400 μ s. The mean (SD) obtained were 7.71 (3.39) at baseline, 107.5 (68.1) following pulse width 400 μ s and 117.9 (67.8) following pulse width 600 μ s. Moreover, when calculating the average percentage change in comparison to the baseline, LDF increased dramatically by 1186% following pulse width 400 μ s and 1552% following pulse width 600 μ s, refer to **Figure 18**.

5.7.3. Echocardiography Assessments

Echocardiography measurements were performed to assess the cardiac output, the percentage of ejection fraction as well diastolic function. Analysis of variance for cardiac output showed a significant increase in LVOT VTI by 6% following the use of electrical stimulation at pulse width 400 μ s and an increase of 4% following pulse width 600 μ s, $p \leq 0.05$. The mean (SD) obtained for LVOT VTI were 21.96 (3.23) for the baseline, 23.27 (3.37) for pulse width 400 μ s and 22.79 (3.06) for pulse width 600 μ s, refer to **Figure 19**. Further analysis of variance showed a non significant change in ejection fraction as well as diastolic function parameters, $p > 0.05$. Parameters remained stable prior to and following electrical nerve stimulation, refer to **Table 8**.

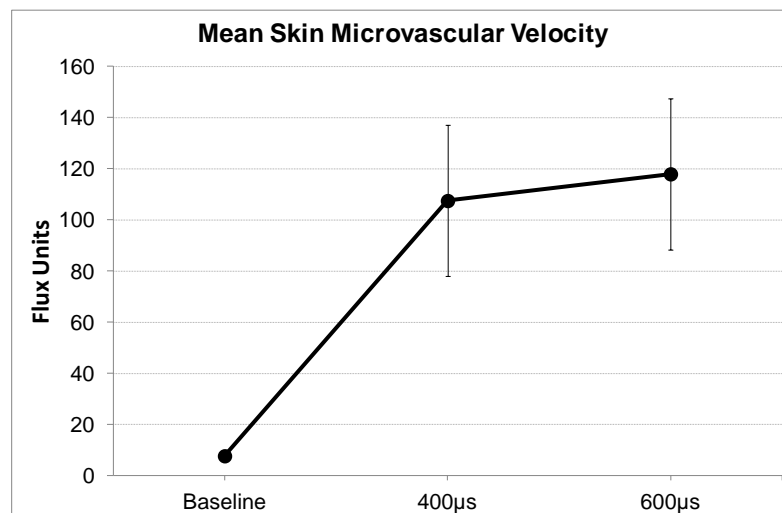


Figure 18: Cardiac Study Mean Skin Microvascular Velocity. Error bars represent 95% confidence interval of the difference from baseline, $p \leq 0.05$.

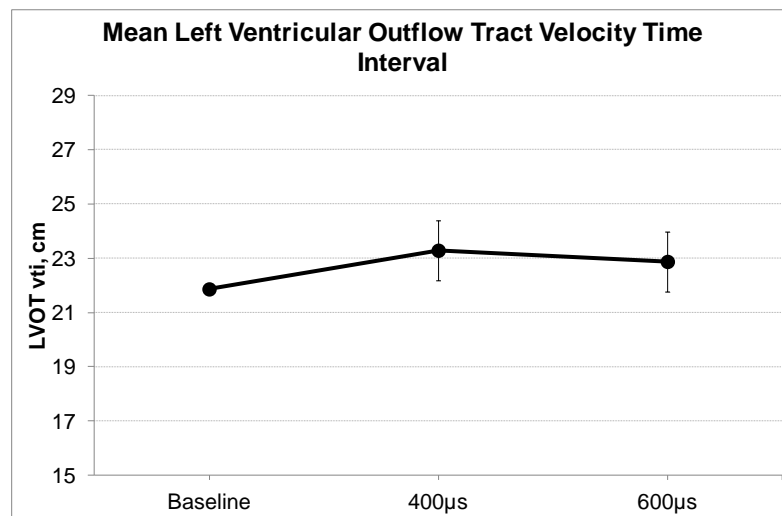


Figure 19: Cardiac Study Mean Left Ventricular Outflow Tract Velocity Time Interval (LVOT VTI). Error bars represent 95% confidence interval of the difference from baseline, $p \leq 0.05$.

Parameters	Baseline Mean (SD)	400µs Mean (SD)	600µs Mean (SD)	P - Value
Ejection Fraction (%)	59.9 (3.72)	60.3 (7.44)	62.6 (4.80)	0.09
LV Diastolic Volume (mL)	117.7 (37.10)	120.2 (32.30)	121.1 (27.86)	0.61
E' Velocity (m/s)	0.80 (0.16)	0.77 (0.12)	0.76 (0.08)	0.29
A' Velocity (m/s)	0.53 (0.13)	0.53 (0.09)	0.54 (0.12)	0.74
Deceleration Time (ms)	187.4 (24.82)	194.1 (31.4)	187.9 (20.28)	0.59
E' Lateral (m/s)	3.76 (5.53)	3.79 (5.58)	3.89 (5.79)	0.75

Table 8: Ejection Fraction and Diastolic Function Measurements prior to and Following Electrical Stimulation.

5.8. Discussion

The aim of the study presented was to investigate the effectiveness of a novel custom built electrical stimulation device (THRIVE) in increasing venous return of the lower limb, which in turn is believed to enhance cardiac performance. Echocardiography was used in this study at different modes to evaluate the effect of applying electrical stimulation at two different pulse widths, 400µs and 600µs. Cardiac parameters measured were ejection fraction, cardiac output as well as diastolic function. Of all of the cardiac parameters assessed, a significant difference was only seen in the cardiac output, $p \leq 0.05$. Cardiac output is the amount of blood ejected by the left ventricle in a time interval

of one minute (Nursecom, 2004). It is measured at the left ventricular out flow tract and is the product of heart rate and stroke volume, which is the amount of blood ejected with each contraction. The parameter used to measure cardiac output is LVOT VTI and the unit of measurement is *cm*. Heart rate and cardiac output have a direct relationship. Thus, as heart rate increases, so does cardiac output. In percentage terms, in comparison to baseline an augmentation in cardiac output by 6% was seen using pulse width 400 μ s and an augmentation of 4% was obtained using pulse width 600 μ s. The observed increase in cardiac output although minimal, it is still in agreement with the results of previous electrical stimulation studies performed by (Faghri et al., 1997). The electrical stimulator used in the study was an eight channel electrical stimulator device, which induced rhythmic pulsatile isometric contractions in the calf and thigh muscles. Following stimulation, a 26% increase in cardiac output was demonstrated. This was compared to a fall by 14% following the use of a sequential compression device (Faghri et al., 1997). The significant increase in cardiac output reported in our study, could be due to the direct activation of the calf muscle pump, which in turn causes the emptying of both venous beds and sinuses. Another reasoning for the increase in cardiac output, could be stress-related and could be due to the exposure to a foreign sensation (electrical stimulation), which may have also resulted in an increase in heart rate. After exposure to the lower pulse width, the increase in cardiac output was 4% following the higher pulse width, which means that the volunteer may have become accustomed to the foreign sensation applied. However, as heart rate was not monitored throughout the study it is difficult to confirm this reasoning.

Ejection fraction (EF), which is a measure of the amount of blood ejected out of the ventricles with each heart beat, was assessed at baseline and following the use of the different pulse widths. Ejection fraction is a predictor of congestive heart failure (Lavine, 2003, Solomon et al., 2005). Although an augmentation of EF by 5% was seen following analysis of the results from our study, however this increase is not statistically significant, $p > 0.05$. Despite that, the increase seen is of interest and requires further investigation on a larger population. It is essential to note here that the subjects in this study are healthy,

thus it is possible that a greater effect may be seen when carrying out the same investigations on patients.

In addition to ejection fraction, assessments of diastolic function parameters were also not statistically significant, $p > 0.05$. A series of diastolic function parameters were measured to assess the left ventricular filling pattern. Parameters include LV diastolic volume, which is the volume of blood available in the left ventricle at the end of diastole and just before systole, E- and A- wave diastolic velocities measured at the mitral valve that reflects the early and late diastolic filling. In addition to the deceleration time (DT) into the left ventricle, and E/E ratio, which is an assessment of the LV filling diastolic pressure. Analysis of the results suggests that electrical stimulation does not alter the filling pattern in the left ventricle.

Furthermore, a statistically significant augmentation was observed when measuring vascular flow parameters both at the arterial and microvascular level, $p \leq 0.05$. Using colour flow duplex ultrasound, arterial volume flow increased by more than 50% following electrical stimulation. Similarly, a lower but a significant increase in arterial peak velocity (24%) was reported. Of interest is the dramatic increase in microvascular velocity by 1186% following pulse width 400 μ s and 1552% following pulse width 600 μ s. There are two possible mechanisms for the increase reported in both the arterial and microvascular measurements. The first mechanism may be that the increased vessel flow provided by the venous valve system when active provides direct auxiliary assistance to the heart, by reducing the pressure difference between inflow and outflow to the ventricle. However, further investigation is required to confirm this mechanism. An alternative mechanism is suggested by the striking increase in micro-vascular velocity as evidenced by the LDF results. This suggests a substantial up-regulation of the use of smaller vessels in the skin and possibly other organs. This represents a large increase in the total available cross sectional area and therefore a presumable drop in the vascular resistance. Hence, reducing the work component of the cardiac cycle.

Findings of this study demonstrate the safety of the device with potential benefits in patients with heart failure; as confirmed by the increase in cardiac output reflected by the

increase in LVOTVTI. The novel device also enhances microcirculatory velocity, which results in the increase of peripheral vascular resistance. This is complimented by a significant enhancement in arterial blood flow volume and velocity. A key limitation in our study was the sample size and nature of the population. Further investigations are required in patients in order to confirm the responses and benefits of electrical stimulation in cardiac patients; particularly those in heart failure.

However, before initialising trials on patients, further research will be performed exploring whether the novel device exhibits any systemic benefits, since studies have demonstrated the enhanced fibrinolytic effects of electrical stimulation (Nicolaides et al., 1972). This will be achieved through performing invasive measurements to examine changes in specific coagulation parameters and explore the exact mode of action.

Chapter 6. The Systemic Effect of a Novel Electrical Stimulation Method

6.1. Introduction

Deep vein thrombosis (DVT) is the formation of a thrombus within the deep veins. Thrombus formation was thought to be only a complication of hospitalisation following surgery, or is associated with late stage terminal illness. However, studies in 1990s banished this myth by demonstrating that thrombus formation occurs not only in hospitalised patients, but also in a high proportion of patients who are not hospitalised nor recovering from major illnesses (Anderson and Spencer, 2003). Death can occur when venous thrombi break off forming pulmonary emboli that pass to the lungs and obstruct the arteries. Thrombus formation usually takes place in areas characterised by a disturbed blood flow in large venous sinuses and in the valve pockets. It has been shown that the majority of thrombi originate in the soleal veins and valve pockets following surgery (Nicolaidis et al., 1971, Nicolaidis et al., 1972). Thrombi also form in vein segments that have been subjected to direct trauma, or inflammatory processes.

As described earlier, a number of risk factors have been well identified, all of which can be associated with an abnormality in one or more of the elements of Virchow's Triad that includes venous stasis, endothelial damage of the veins and hypercoagulability. Some factors are considered strong risk factors that require prophylaxis such as general surgery, orthopaedic surgery, spinal cord injury, major trauma, malignancy, myocardial infarction, congestive heart failure and respiratory failure. Others are recognised as weak factors such as advanced age, hormone therapy, smoking and obesity that may require prophylaxis if present in combination with other risk factors (Anderson and Spencer, 2003). Therefore, it is essential to assess the strength of individual risk factors and the combined effects of risk factors prior to selecting the appropriate prophylaxis.

Despite the great advances in pharmacological prophylaxis, the bleeding risk associated with their use and the constant need of monitoring is a major hurdle. DVT still remains a

major public health problem; hence, improving ways of prevention is the key to tackling this issue.

The underlying study referred to as Thromboembolic Prevention via Electrical Stimulation (THRIVE) investigated the use of a novel method for enhancing blood flow in the lower limb using electrical nerve stimulation. An increase in lower limb blood flow improves venous return and thereby reduces venous stasis. Since stasis is one of the compounding factors in the development of deep vein thrombosis, the risk of developing thrombosis should theoretically be proportionally reduced. The technique investigated may therefore, prove to be useful in preventing and reducing the incidence of deep vein thrombosis in both medical and surgical patients together with passengers undertaking significant travel.

The proposed technique involves activation of the venous muscle pumps of the lower leg (foot and calf pump) via electrical stimulation of the lateral and/or medial popliteal nerves located within the popliteal fossa. This involves placing electrodes over the nerves in the lower thigh and locations anatomically distant from the calf and foot. In other studies, activation of the muscle pumps has been achieved by direct stimulation of the calf itself by placing the electrodes over the muscles themselves. In preliminary investigations in our laboratory, the technique has been shown to be effective in stimulating lower limb musculature contraction measured by strain-gauge plethysmography. Contraction of the lower limb and foot muscles has been demonstrated to enhance both blood flow velocity and flow volume in the lower limb as measured in dorsal foot veins by plethysmography and in the femoral vein using duplex ultrasound (Tucker et al., 2010).

6.2. Objectives

The key objective of this study was to investigate the effect of the electrical stimulation device (THRIVE) on specific blood coagulation factors. Secondary objectives were to evaluate the effectiveness of the THRIVE device in enhancing lower limb blood flow by assessing blood flow velocity and volume changes at the arterial and venous level using

colour flow duplex ultrasound and laser Doppler flowmetry. Furthermore, to assess the safety of the electrical stimulation applied, by monitoring oxygen saturation levels as well vital signs throughout the study period. In addition to this, to assess the acceptance and tolerance of healthy volunteers to the stimulation device applied using discomfort questionnaires.

6.3. Study Approval

The study sponsored by Barts and The London School of Medicine and Dentistry and funded by Sky Medical Technology Limited was approved by the National Research Ethics Service Committee (REC Number: 05/0408/14 Brent) refer to Appendix 4.

6.4. Materials & Methods

6.4.1. Volunteers

Ten healthy volunteers (9 Males, 1 Female) were recruited by advertisement to staff and students at Barts and the London, Queen Mary University, Barts and The London NHS Trust and to the general community. The demographic data are displayed in **Table 9** below. Of the ten subjects recruited, 9 were White Europeans and 1 was from another ethnic origin. All subjects complied with the study protocol. The specific inclusion and exclusion criteria are outlined in **Table 10** and **Table 11**.

Volunteers were instructed to have a light breakfast, avoiding fatty foods and caffeine containing products. They were also asked to refrain from vigorous exercise from the previous evening onwards. All volunteers attended The Ernest Cooke Clinical Microvascular unit at St Bartholomew's Hospital where assessments were performed. Upon arrival volunteers were provided with information sheets (Appendix 5) and written informed consent for screening and study participation was obtained prior to the study, see Appendix 6 & 7. Each volunteer had two visits; the first visit was to conduct a stimulation study where an electrical stimulation device was applied. The second visit was to carry out a control study where the same investigations were repeated without stimulation.

Parameters	Mean (SD)	Median[Range]
Sex; M: F	9:1	
Age; Years	34.6 (6.88)	35 [26 – 45]
Body Mass Index (BMI)	25.3 (2.86)	24.8 [20.1- 30.2]

Table 9: Demographic Data for THRIVE Study Volunteers.

Health	Good general health
Age	Between 18 – 65
Medical History	No abnormal findings, absence of DVT and haematological disorders
Body Mass Index (BMI)	Between 18 – 34
Drugs	No history of drug abuse (including alcohol) licit or illicit
Medication	No medication usage during 30 days preceding the study or during the course of the study

Table 10: Inclusion Criteria for THRIVE Study.

6.4.2. Electrical Nerve Stimulation Device (THRIVE)

During the stimulation study, an in house custom built electrical stimulation device was applied transcutaneously to the common peroneal nerve in the popliteal fossa of one leg and compared to an un-stimulated contralateral control leg. The stimulation device used in this study is similar to that used in the cardiac study, which consists of two electrodes connected to a stimulator box refer to

Figure 20. The electrical stimulation device was used at an amplitude of 25mA and a frequency of 3Hz with a pulse width of 600µs. Those settings were chose in relation to an optimum stimulation setting obtained from previous study results (Tucker et al., 2010). The stimulation was applied for 5 minutes every 15 minutes followed by a 10 minute recovery phase, to allow vascular re-equilibration.

Health	Organ dysfunction, any clinically significant deviation from normal in the physical determinations
Age	< 18 or > 65 years
Medical History	Hematological disorders, previous DVT/PE, varicose veins or lower limb ulceration, musculoskeletal disorders, recent surgery and recent trauma to lower limb; and history of gastrointestinal, hepatic, renal, cardiovascular, endocrine, neurological, dermatological, rheumatological, metabolic (including diabetes), psychiatric, or systemic disease judged to be significant
Body Mass Index (BMI)	Chronic obesity (BMI >34 kg/m ²)
Pregnancy	Positive pregnancy test
Ankle Brachial Pressure Index (ABPI)	Peripheral arterial disease (ABPI <0.9)
Medication	Consumption of any medication in the previous 30 days
Blood	Contraindication to blood sampling, Donation of blood 8 weeks preceding the study
Participation in other clinical study	Participation in any clinical study during the 8 weeks preceding the active period of the study

Table 11: Exclusion Criteria for THRIVE Study.



Figure 20: Custom Built Electrical Stimulation Device. The Electrical stimulation device (THRIVE) was applied over the peroneal nerve located at the back of the knee to subjects seated in an airline seat.

6.5. Study Procedure

6.5.1. Screening Evaluation

After explaining the study procedure and following obtaining informed consent, volunteers were screened prior to starting the study. The screening evaluation included a medical history, physical examination together with vital signs (blood pressure and heart rate) and colour flow duplex ultrasound of the lower legs to exclude bilateral DVT. The ultrasound examination involved examining the deep veins (femoral, popliteal, gastrocnemial, soleal, posterior tibial, and peroneal) for vessel wall irregularities that might suggest a history of DVT. Furthermore, the superficial venous system (greater saphenous veins and lesser saphenous veins) were also examined for valvular incompetence (reflux) to exclude the presence of superficial vein thrombophlebitis.

6.5.2. Study Methodology

The study was carried out in a quiet, stable, draught free environment, where both the temperature and humidity were controlled ($24 \pm 1^{\circ}\text{C}$, relative humidity 30 – 40%). Following screening, eligible volunteers were cannulated by an anaesthetist in the vein at three sites: bilaterally in the foot (dorsum or medial malleolar region) and one arm (left arm). Volunteers lightly clad in shorts, then sat in an airline seat with their legs bent at the knees for a period of 4 hours. Changes in lower limb blood flow were then evaluated during the 4 hour study period using laser Doppler flowmetry (LDF), Colour Flow Duplex Ultrasound. Pulse oximetry was also used to measure the oxygen saturation and heart rate. Blood pressure was also monitored during the study period using a digital blood pressure monitor (UA- 767PC, A&D Tokyo, Japan).

6.5.2.1. Laser Doppler Flowmetry (LDF)

Microcirculatory blood flow was assessed using Laser Doppler Perfusion & Temperature Monitor DRT4; Moor Instruments Ltd, UK. Probes were attached bilaterally to the foot for each volunteer. Following zero calibration and stabilisation of the LDF signals, skin blood flow was recorded for 5 minutes at baseline and at the end of the stimulation

period, while the device is still active. LDF signals recorded for 5 minutes, were then processed as mentioned earlier by a computer analysis system, CED 1401 using Spike 2 software (Cambridge Electronic Design Ltd., Cambridge UK), which averages the recordings, thereby reducing the effect of artefact. From the LDF signals generated, the average values of LDF signals were generated. Adjusted LDF were then calculated by deducting the zero calibration values from the actual LDF measurements.

6.5.2.2. Colour Flow Duplex Ultrasound

Colour flow duplex ultrasound (Phillips HDI 5000) was used to evaluate the effect of the electrical stimulation on blood flow in the superficial and deep venous system throughout the study. Ultrasound assessments of the arterial and venous femoral blood vessels were performed by an accredited vascular ultrasonographer at the end of each time interval i.e. Baseline, 1, 2, 3 and 4 hours and recorded manually in the subject's case report form. The parameters measured include blood volume flow, peak velocity together with vessel wall diameter.

6.5.2.3. Pulse Oximetry

The percentage of haemoglobin saturated with oxygen, together with heart rate was measured using Pulse Oximetry. Readings were obtained through the use of a light sensor which contains two sources of light (red and infrared). Light is absorbed by haemoglobin and is transmitted through the tissue to the photo detector. The amount of light transmitted through the tissues is then converted to a digital value representing the percentage of oxygen saturation of the haemoglobin. Normal oxygen saturation values usually lie between 96-99% in healthy individuals, however values down to 90% are also normal but rarely occur (Minolta, 2006). Measurements using pulse oximeter (Datex Ohmeda 3900P, TruTrak+[®]) were made by placing a probe on the pulp of the second left toe of each volunteer. Percentages of oxygen saturation (SPO₂) as well as, heart rate readings were then recorded manually from the pulse oximeter device immediately after the stimulation period. Pulse oximetry recording were not performed during the stimulation period, as unstable reading were given as a result of muscular contraction.

6.5.2.4. Blood Collection

Blood was withdrawn from the three sites cannulated (bilaterally at each leg and left arm) at the start of the investigation and serially during the 4 hour study period. Approximately 15mL of blood was collected at each site at each time point. Patency of each cannula was maintained by a saline flush preceding and following each collection. Prior to blood collection, 1-2mL of blood was withdrawn and discarded, as this represents blood and saline, which has been resident in the cannula or in close proximity to the cannula tip.

As all parameters analysed were coagulation parameters, blood was collected into a 3.2% 0.105M sodium citrate light blue top vacutainer tube (ratio 9:1) according to the Flow Chart presented in Appendix 8. Plasma was prepared by centrifugation for 10 minutes at 2500g at room temperature. The platelet poor plasma that was aliquoted into pre-labelled 2mL cryovials was immediately frozen. The cryovials were then placed in pre-labelled cardboard boxes and stored at -80⁰C until further analysis (Appendix 9).

6.5.2.5. Assessment of Clotting Time

As prolonged sitting is usually associated with clot formation in the lower limb, it was essential to analyse several parameters that are closely associated with the clotting time of blood throughout the different time intervals. This was carried out using two techniques: Medtronic Automated Coagulation Timer System (ACT) and Rotational Thromboelastometry (Rotem®). ACT is a microprocessor controlled electrochemical coagulation instrument, designed to determine coagulation endpoints in whole blood samples. Freshly drawn whole blood (2mL) was analysed at each time interval according to the standard operating instructions (Appendix 10). The clotting time of the whole blood was measured immediately following collection by placing 0.2mL of blood in a cartridge channel that has an activating agent (Kaolin). ACT detects clot formation by measuring the “falling time” of a plunger that is contained in each cartridge channel. The plunger-flag falls rapidly in an un-clotted sample, but as the fibrin forms, it delays the rate of descent, which is detected by a photo optical system. Simultaneous duplicate tests are being performed by using the provided two channels cartridges. The results for each channel are being displayed in seconds following the completion of the test.

Rotational thromboelastometry (Rotem®) is another technique used to assess blood coagulation. However, unlike ACT, thromboelastometry attempts a detailed graphical representation of the fibrin polymerisation process beginning with initial clot formation, followed by clot acceleration phase and ending with clot lysis. The Rotem® device consists of four measurement channels that allow simultaneous assays to be performed measuring the extrinsic coagulation pathway through ‘Extem Test’ and the intrinsic pathway through ‘Intem Test’. In the Extem coagulation is activated by tissue factor. This leads to the initiation of clot formation within 70 seconds. Thus, clot formation can be assessed within 10 minutes. In contrast, with the ‘Intem Test’, coagulation is activated via the contact phase (as in the ACT). Rotem® device is connected to a PC for automatic analysis as well as an electronic pipette for interactive test operation. Approximately 240 µL of citrated blood sample was placed in a disposable cuvette with the addition of the appropriate reagent according to the standard operating procedure for Rotem® (Appendix 11). A cylindrical pin is immersed, which in turn is connected to an optical detector. As soon as the blood clots, the clot restricts the rotation of the pin. The signal of the pin suspended in the blood sample, is then graphically displayed as a trace onto the PC, refer to **Figure 21**. The key parameters measured at different stages of clot formation include:

Clotting Time (CT): the time from the start of the measurement until initiation of clotting; units of measurement is seconds.

Clot Formation Time (CFT): is the time from initiation of clotting until a clot firmness of 20 mm is detected; units of measurement is seconds.

Maximum Clot Firmness (MCF): is the firmness of the clot, measured in mm.

Maximum Lysis (ML): is the percentage reduction of clot firmness after MCF (breakdown of the clot)

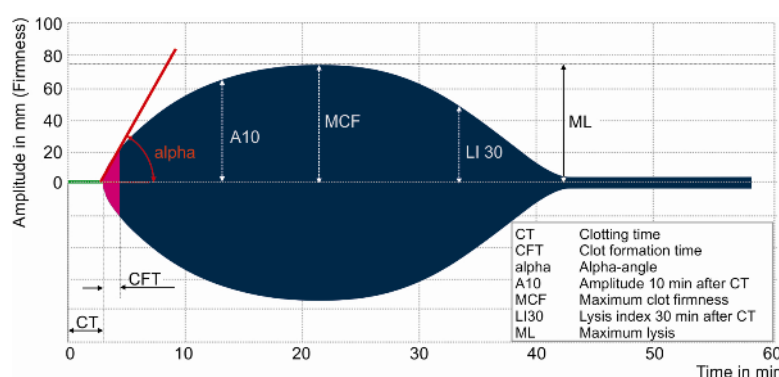


Figure 21: A Two- Sided Rotem® Trace Displaying The Important Parameters Measured Throughout Time (Calatzis et al.)

6.5.2.6. Assessment of Coagulation Factors

Coagulation factors were assessed during the 4 hour sitting period to investigate the haematological effects of the electrical stimulation. The coagulation factors analysed were, Tissue Plasminogen Activator (t-PA) Antigen, von Willebrand Factor (vWF) and 6-Keto Prostaglandin F1 alpha. Assessment of those factors was performed using Enzyme Linked Immunosorbent Assay (ELISA).

6.5.2.6.1. Tissue Plasminogen Activator Antigen Assay

Tissue plasminogen activator the primary initiator of fibrinolysis in the vascular system plays an important role in regulating the formation and removal of thrombi. Therefore, a deficiency in tPA can result in a reduction of the capacity to degrade a clot, hence predispose to thrombosis. Levels of tPA in the plasma were measured using a tPA ELISA Kit (IMUBIND® tPA ELISA Kit, American Diagnostica Inc), refer to Appendix 12 for tPA ELISA Protocol. Frozen aliquots of the platelet-poor plasma were thawed and assayed using the tPA ELISA kit. 20µL of sample was added in duplicates to microwells coated with polyclonal anti human tPA antibody. The tPA antibody captures the tPA antigen during the incubation period. Unbound materials were then washed away and bound tPA antigen was identified using peroxidase enzyme substrate. The reaction was then terminated by the addition of 50µL of sulphuric acid, which turns the colour from yellow to orange. Absorbance of the solution was then measured at 490 nm using ELISA spectrophotometer reader (Wellcozyme, Wellcome Diagnostics, Analytical Services International Ltd, UK). A standard curve was then constructed by plotting the mean

absorbance value measured with each tPA standard versus its corresponding concentration. The standard curve was then used to extrapolate the tPA antigen concentrations of the plasma samples from the ELISA assay using the Windows Excel Program. To maintain quality control, plasma samples were selected and repeated with each new assay.

6.5.2.6.2. von Willebrand Factor Assay

Von Willebrand factor is a possible indicator of endothelial dysfunction, where raised levels are associated with increased thromboembolic events. Thus, it was essential to measure quantitatively vWF levels in the plasma of participants over time at the three different sites, in order to assess the effectiveness of the electrical stimulation in the prevention of thrombus formation. Frozen, stored aliquots of the platelet-poor plasma were thawed and analysed by Enzyme Linked Immunosorbent Assays (ELISA) vWF kit (IMUBIND[®] vWF ELISA Kit, American Diagnostica Inc). Refer to Appendix 13 for vWF ELISA Protocol. 100µL of diluted sample was added in duplicates to microwells coated with polyclonal goat antibody. The antibody captures the vWF during the incubation period. After washing the unbound material, peroxidase conjugate detection antibody was added and incubated for a further period. Following incubation, wells were washed thoroughly again. 100µL of substrate was then added and the plate was further incubated for a short period (10 minutes) to allow blue colour to develop. The enzymatic reaction was then terminated by the addition of 50µL of sulphuric acid to each well, which changed the colour from blue to yellow. Absorbance was then measured at 450nm immediately. A standard curve based on a quadratic equation was plotted using Windows Excel Program and used to interpolate concentrations of vWF in the plasma sample. To maintain quality control, plasma samples were selected and repeated with each new assay.

6.5.2.6.3. Prostacyclin Assay

Prostacyclin, a major product produced by endothelial cells is responsible for inhibiting platelet aggregation. However, as prostacyclin has a short half-life in plasma, the production of PGI₂ can be measured indirectly, through measuring the levels of its stable

product, 6 keto PGF1 F1 α . To do this, 50mg of indomethacin was first dissolved in 3.5mL of H₂O to yield a final concentration of 40mM. 100 μ L of the indomethacin solution prepared earlier was then added to citrated blood prior to centrifugation at 2500g for 10 minutes to block the metabolism of prostacyclin (Webberley et al., 1993). The ELISA used to assay 6 keto PGF1 α is based on a competitive technique. The ELISA kit used (Neogen Corporation), operates on the basis of competition between the enzyme conjugate and 6 keto PGF1 α in the sample for a limited number of binding sites on the antibody coated plate. 6 keto PGF1 α was first extracted using solid phase extraction method. Following extraction, 50 μ L of the diluted enzyme conjugate was then added to each well and incubated for an hour. Following incubation, the plate was washed thoroughly using diluted wash buffer to remove any unbound material. 150 μ L of substrate was then added to each well to detect the bound enzyme conjugate. Following an additional incubation period (30 minutes), colour is detected. The extent of colour development is inversely proportional to the amount of 6 keto PGF1 α in the sample. For instance, the absence of 6 keto PGF1 α in the sample will result in a bright blue colour, whereas its presence will result in a decreased or no colour development. The enzymatic reaction was then terminated by the addition of 100 μ L of stop solution to each well. Absorbance was then read at 450nm; refer to Appendix 14 for a detailed description of the protocol. From the absorbance values obtained a four parameter logistic calibration curve was constructed by plotting absorbance versus concentration in ng/mL using Windows Excel Program. The four parameter logistic curve constructed was based on the following equation:

$$Y = NSB + \frac{(TOP - NSB)}{1 + \left(\frac{x}{ED50}\right)^{slope}}$$

where:

x = Concentration

NSB = The lower value of the curve (non-specific binding)

TOP = The upper value of the curve (maximal binding)

ED50 = The midpoint value of the curve

Slope = Slope of the curve

Y= Absorbance value

6.5.2.6.4. Endogenous Thrombin Generation and D-dimer Assay

Further coagulation marker assessments were carried out by measuring levels of D-dimer, the final degradation product of fibrinolysis and Endogenous Thrombin Potential (ETP), which is an indicator of the thrombin generating capacity. Both analysis were performed by an external laboratory (Haemostasis Laboratory, Blood Science Department, NHS, London).

6.5.2.7. Tolerance and Acceptance

Following electrical stimulation, the volunteers were provided with a discomfort questionnaire. Discomfort was compared to a blood pressure cuff inflated around the upper arm. Volunteers were asked to rate their level of discomfort using a Visual Analogue Scale (VAS), by marking the level of the perceived pain using a 100mm line, marked at one end “no sensation” and at the other end “severe discomfort”. Verbal Rating Score (VRS) was also used to categorise the rating of the discomfort pain as shown below, refer to Appendix 15 for a sample of VAS and VRS.

- 1 = no sensation (other than muscles tensing and relaxing)
- 2 = minimal sensation
- 3 = mild discomfort
- 4 = moderate discomfort
- 5 = severe discomfort

6.6. Statistical Analysis

All assessments obtained during the stimulation and control study were acquired at baseline and after 1, 2, 3 and 4 hours. As mentioned in the previous chapter, the data were analysed untransformed. As all subjects were healthy and were not expected to have any abnormal pathological findings, the baseline values obtained for each assessment were therefore, used as a reference range. Statistical analysis was performed using Minitab 16 software (Minitab Ltd, UK) as in the previous chapter. Results obtained during the stimulation study were compared to those obtained during the control study at each time interval with the baseline values acting as reference. The data shown below represent the mean of the data obtained from the ten volunteers and are presented as mean (SD).

6.7. Results

6.7.1. Colour Flow Duplex Ultrasound Assessments

Colour flow duplex ultrasound assessments to the arterial and venous femoral blood vessel were performed at each time interval (Baseline, 1, 2, 3 and 4 hours) refer to **Figure 22** & **Figure 23**. Selected parameters were investigated, these include: blood volume flow (mL/min), peak maximum velocity (cm/sec) and femoral vessel wall diameter (mm).

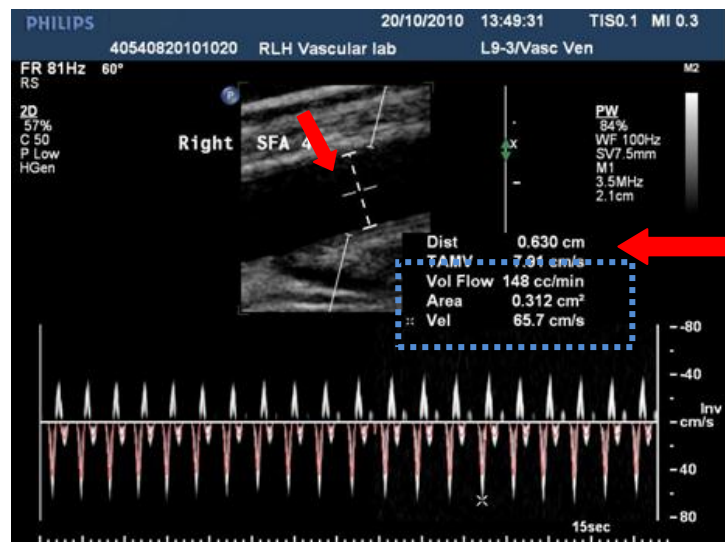


Figure 22: Typical Colour Duplex Ultrasound Waveform of the Femoral Artery following 4 Hours of Stimulation. Red arrow highlights the femoral arterial diameter measured. Time Averaged Mean Velocity (TAMV) and Cross Sectional Area (Area) are then calculated by the software to produce the Volume Flow (Vol Flow) and Maximum Peak Velocity (Vel), outlined by the blue box.

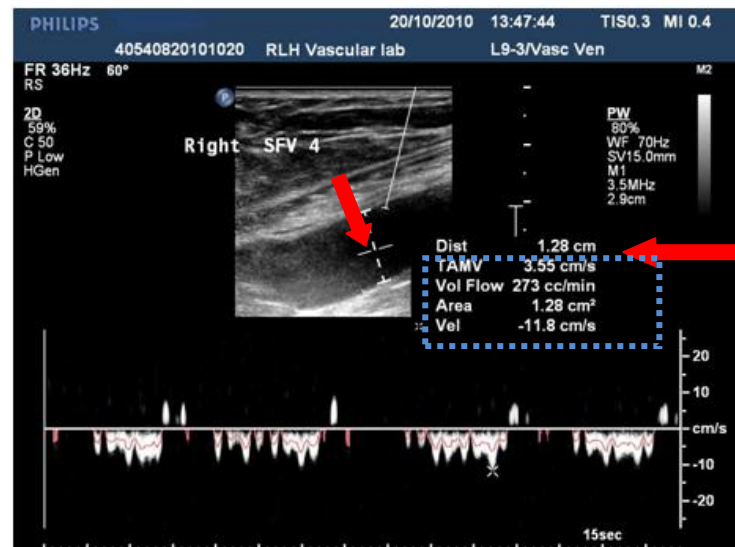


Figure 23: Typical Colour Duplex Ultrasound Waveform of the Femoral Vein following 4 Hours of Stimulation. Red arrow highlights the femoral vein diameter measured. Time Averaged Mean Velocity (TAMV) and Cross Sectional Area (Area) are then calculated by the software to produce the Volume Flow (Vol Flow) and Maximum Peak Velocity (Vel), outlined by the blue box.

6.7.1.1. Mean Peak Maximum Velocity Assessments

Analysis of the control study data using linear models, demonstrated a non significant difference in femoral velocity both at the arterial and venous level, $p > 0.05$ refer to **Figure 24**. The mean (SD) reported for the arterial and venous femoral blood vessel in the control study are summarised in **Table 12**.

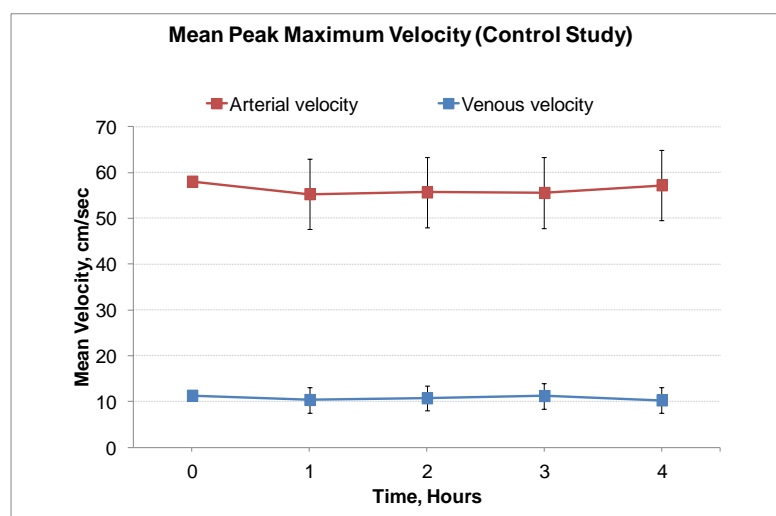


Figure 24: Mean Peak Maximum Velocity throughout the Control Study. Error bars represent 95% confidence interval of the difference from baseline; $p > 0.05$.

Time	Arterial Velocity (cm/sec) Mean (SD)	Venous Velocity (cm/sec) Mean (SD)
Baseline	58.01 (9.48)	11.28 (2.62)
1 Hour	55.27 (12.69)	10.35 (4.34)
2 Hours	55.66 (9.19)	10.72 (2.76)
3 Hours	54.54 (8.22)	11.21 (4.33)
4 Hours	57.21 (7.41)	10.26 (2.30)

Table 12: Mean Peak Maximum Velocity Measurements obtained throughout the Control Study, $p > 0.05$.

Similarly following stimulation, no significant difference was reported at the arterial vessel, $p > 0.05$ refer to **Figure 25**. The mean (SD) measurements reported at the arterial vessel are summarised in **Table 13**. In contrast, a highly significant difference was reported at the venous vessel, $p \leq 0.001$ as shown in **Figure 25**. The mean (SD) venous velocity measurements reported were 10.70 cm/sec (4.32) at baseline, which then increased by 158% to 24.09 cm/sec (8.30) after 1 hour and further increased by 169% with a mean (SD) of 26.82 cm/sec (6.57) after 2 hours. Similarly, an increase in venous velocity by 221% at 3 hours with a mean (SD) of 30.07 cm/sec (13.32) was demonstrated. This was followed by a small fall at 4 hours as indicated by an average percentage change of 162% and mean (SD) of were 25.67 cm/sec (5.54).

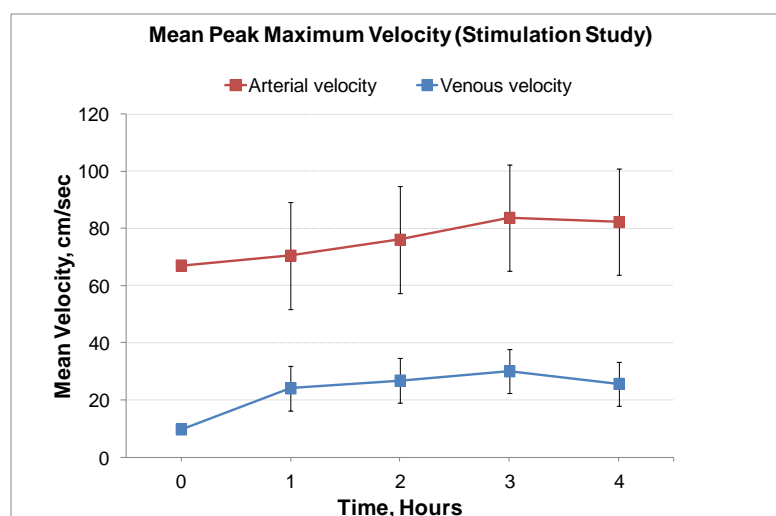


Figure 25: Mean Peak Maximum Velocity throughout the Stimulation Study. Error bars represent 95% confidence interval of the difference from baseline; venous velocity $p \leq 0.001$, arterial velocity $p > 0.05$.

Time	Arterial Velocity (cm/sec) Mean (SD)
Baseline	69.92 (21.32)
1 Hour	70.47 (28.50)
2 Hours	76.04 (16.47)
3 Hours	83.69 (13.03)
4 Hours	82.23 (15.56)

Table 13: Mean Arterial Peak Maximum Velocity Measurements obtained throughout the Stimulation Study, $p > 0.05$.

6.7.1.2. Mean Blood Volume Flow Assessments

Analysis of blood volume flow measurements obtained throughout the control study showed a significant drop in the arterial volume flow in comparison to the baseline, $p \leq 0.05$. This is in comparison to a non-significant difference in the venous blood vessel; $p > 0.05$ refer to **Figure 26**. The mean (SD) measurements obtained for both arterial and venous blood volume flow are summarised in **Table 14**. Calculating the average percentage change from baseline showed a drop in arterial volume flow by 21% after 1 hour, a small drop of 3% was then reported after 2 hours, followed by a greater drop of

14% after 4 hours that was interestingly followed by a small increase by 3% after 4 hours.

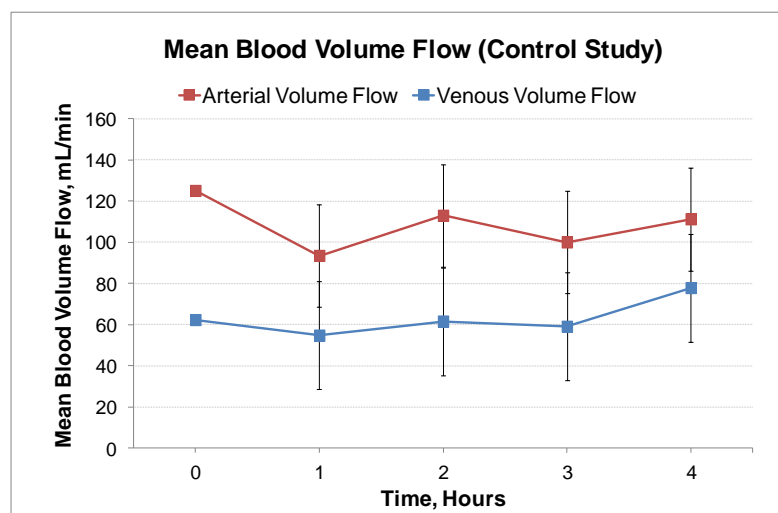


Figure 26: Mean Blood Volume Flow throughout the Control Study. Error bars represent 95% confidence interval of the difference from baseline; venous volume flow: $p > 0.05$, arterial volume flow: $p \leq 0.05$.

Time	Arterial Volume Flow (mL/min) Mean (SD)	Venous Volume Flow (mL/min) Mean (SD)
Baseline	125 (47.4)	62.23 (33.4)
1 Hour	93.4 (35.2)	54.7 (38.7)
2 Hours	112.9 (40.4)	61.5 (37.7)
3 Hours	100.0 (45.5)	59.0 (44.3)
4 Hours	111.20 (29.30)	77.0 (74.4)

Table 14: Mean Blood Volume Flow Measurements obtained throughout the Stimulation Study. Arterial Volume Flow $p \leq 0.05$, Venous Volume Flow $p > 0.05$.

Further analysis using linear models for the stimulation study showed a significant difference in mean blood volume flow measurements at both the venous and arterial levels. In comparison to arterial blood flow results obtained in the control study, the mean arterial volume flow levels increased following stimulation, $p \leq 0.05$ refer to **Figure 27**. The mean (SD) reported were 176.6 mL/min (65.6) at baseline, that increased substantially by 125% after 1 hour 288.7 mL/min (127.2), a slight increase by 62% was then reported at 2 hours 237.3 mL/min (81.7). Arterial volume flow then increased by

99% at 3 hours followed by 87% at 4 hours, with the mean (SD) values being 259.4 mL/min (71.9) and 253 mL/min (100.1) for 3 and 4 hours.

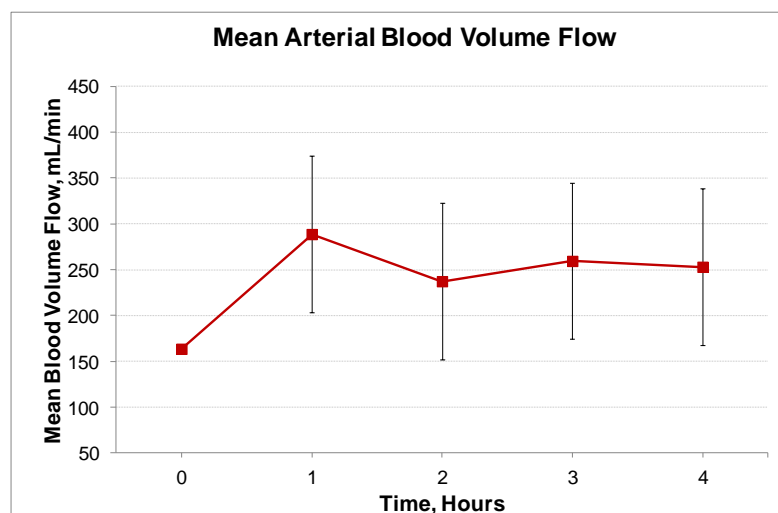


Figure 27: Mean Arterial Blood Volume Flow throughout the Stimulation Study. Error bars represent 95% confidence interval of the difference from baseline; $p \leq 0.05$.

Similarly, a highly significant increase was reported when measuring the venous blood volume flow in the femoral vessel, $p \leq 0.001$ refer to **Figure 28**. The mean (SD) were 59.4 mL/min (41) at baseline, which then increased dramatically after 1 hour to 233.76 mL/min (114.5) followed by 224.6 mL/min (76.3) after 2 hours. A further increase was then reported at 3 hours and the mean (SD) values were 253 mL/min (86.8) that was followed by a slight fall to 223 mL/min (76.9) after 4 hours. When considering the average percentage change in comparison to baseline for the venous volume flow measurements, a 489% increase was reported after 1 hour that further increased substantially by 374% after 2 hours. The increase was the highest at 3 hours where the average percentage change reported was 471% followed by an increase by 405% increase at 4 hours.

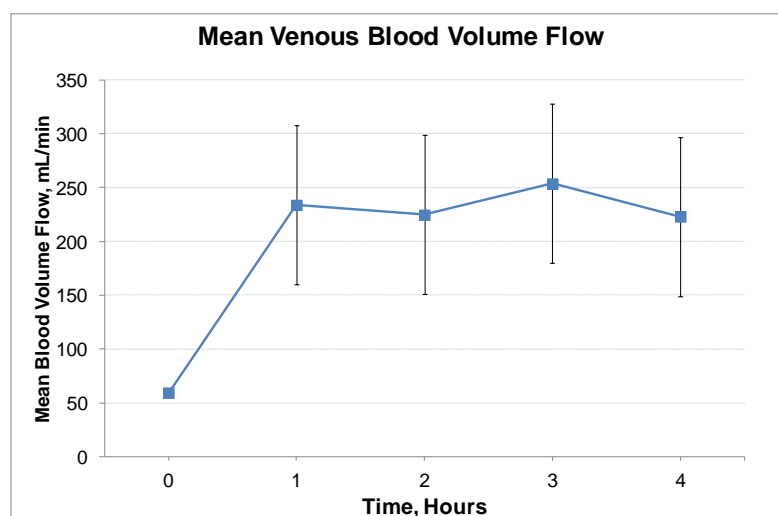


Figure 28: Mean Venous Blood Volume Flow throughout the Stimulation Study. Error bars represent 95% confidence interval of the difference from baseline; $p \leq 0.001$.

6.7.1.3. Femoral Vessel Wall Diameter Assessments

No significant difference in mean vessel diameter at both the venous and arterial level was reported in the control and stimulation study, $p > 0.05$ refer to **Figure 29**, **Figure 30** and **Figure 31**. Measurements remained equally stable throughout the study. The mean (SD) obtained in both studies are summarised in **Table 15** and **Table 16**.

Time	Arterial Blood Vessel (mm) Mean (SD)	Venous Blood Vessel (mm) Mean (SD)
Baseline	5.91 (2.01)	15.17 (22.04)
1 Hour	5.76 (2.02)	16.94 (28.03)
2 Hours	5.46 (2.17)	13.50 (17.76)
3 Hours	5.70 (2.09)	12.79 (14.63)
4 Hours	5.66 (1.94)	13.83 (17.51)

Table 15: Vessel Wall Diameter throughout the Control Study, $p > 0.05$.

Time	Arterial Vessel Diameter (mm) Mean (SD)	Venous Vessel Diameter (mm) Mean (SD)
Baseline	1.27 (1.89)	1.91 (2.92)
1 Hour	1.27 (1.90)	1.71 (2.38)
2 Hours	1.23 (1.89)	1.76 (2.65)
3 Hours	1.12 (1.52)	1.78 (2.70)
4 Hours	1.17 (1.72)	1.74 (2.60)

Table 16: Vessel Wall Diameter throughout the Stimulation Study, $p > 0.05$.

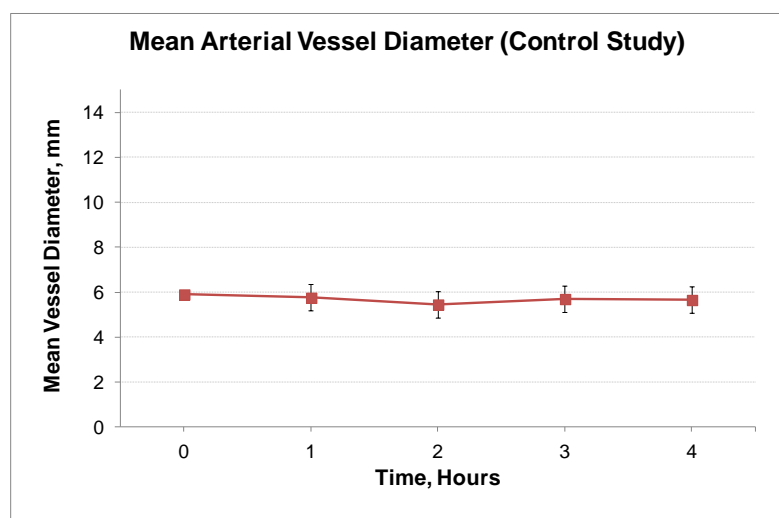


Figure 29: Mean Arterial Vessel Wall Diameter throughout the Control Study. Error bars represent 95% confidence interval of the difference; $p > 0.05$.

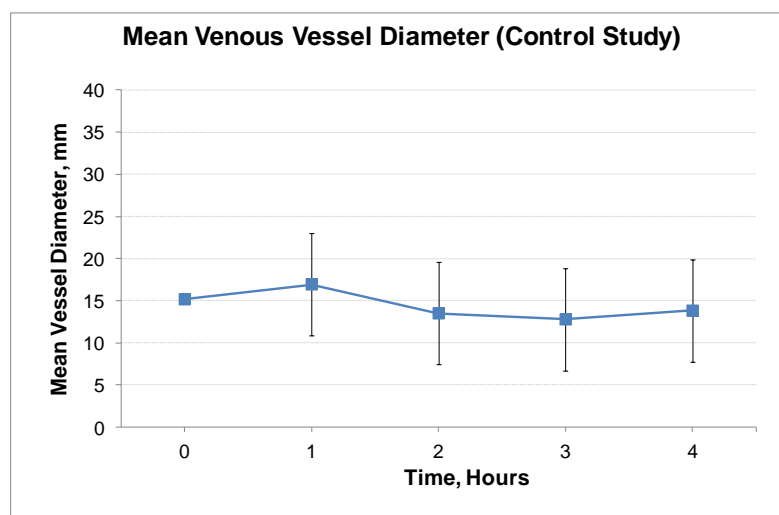


Figure 30: Mean Venous Vessel Wall Diameter throughout the Control Study. Error bars represent 95% confidence interval of the difference from baseline; $p > 0.05$.

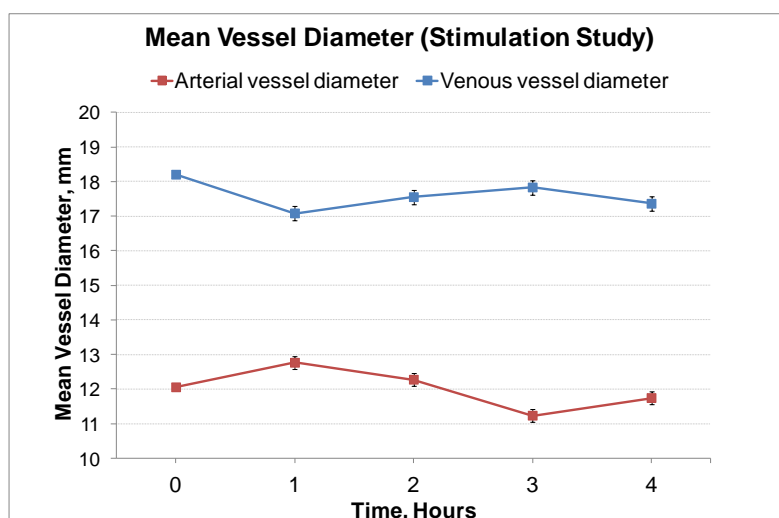


Figure 31: Mean Vessel Wall Diameter throughout the Stimulation Study. Error bars represent 95% confidence interval of the difference from baseline; $p > 0.05$.

6.7.2. Skin Microcirculatory Assessments

Analysis of the data obtained using laser Doppler flowmetry (LDF) showed a non significant difference in the control study at both sites measured (right leg and left leg), $p > 0.05$, refer to **Figure 32**. The mean (SD) obtained at both sites are summarised in **Table 17**.

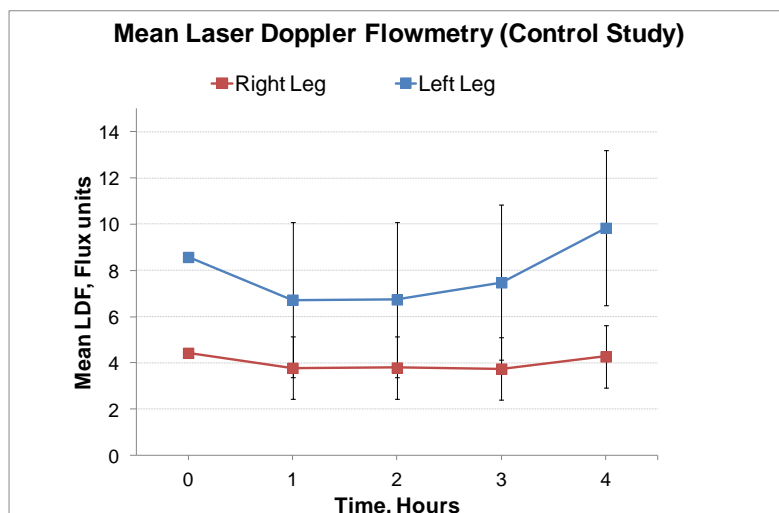


Figure 32: Mean LDF Measurements throughout the Control Study. Error bars represent 95% confidence interval of the difference from baseline; $p > 0.05$.

Time	LDF Right Leg (Flux Units) Mean (SD)	LDF Left Leg (Flux Units) Mean (SD)
Baseline	4.43 (5.83)	8.58 (2.45)
1 Hour	3.78 (5.52)	6.73 (2.13)
2 Hours	3.79 (5.76)	6.74 (2.01)
3 Hours	3.75 (6.40)	7.48 (3.01)
4 Hours	4.27 (6.62)	9.84 (6.45)

Table 17: LDF Results at the Right and Left Leg.

However, analysis of the data obtained from the stimulation study showed a highly significant difference in the stimulated leg as compared to passive, $p \leq 0.001$. Mean LDF values remained stable throughout the study at the passive leg; $p > 0.05$ refer to **Figure 33**. The mean (SD) recorded throughout the stimulation study is summarised in **Table 18**.

Time	LDF PassiveLeg (Flux Units) Mean (SD)	LDF Stimulated Leg (Flux Units) Mean (SD)
Baseline	7.35 (6.14)	4.66 (5.09)
1 Hour	9.36 (6.52)	73.6 (62.4)
2 Hours	7.74 (4.40)	70.4 (62.3)
3 Hours	8.77 (6.42)	73.9 (56.5)
4 Hours	7.79 (5.99)	75.8 (54.1)

Table 18: LDF Results for the Passive and Stimulated Legs.

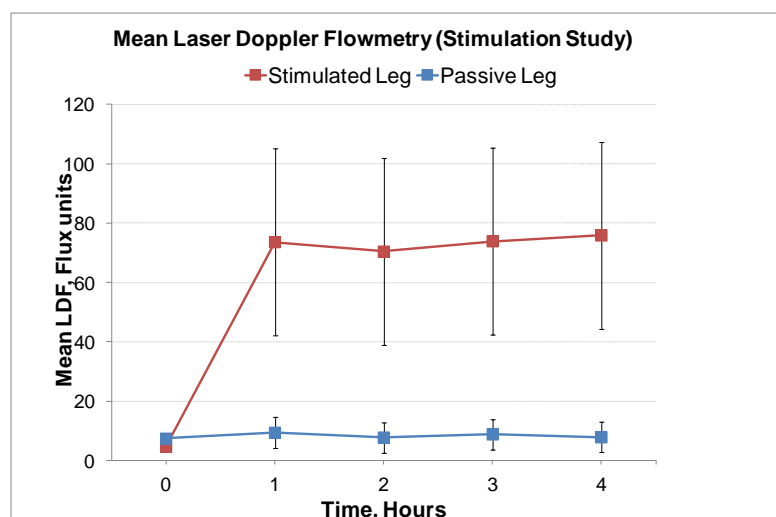


Figure 33: Mean LDF Measurements throughout the Stimulation Study. Error bars represent 95% confidence interval of the difference from baseline; passive leg: $p > 0.05$, stimulated leg: $p \leq 0.001$.

6.7.3. Clotting Time Assessments

Assessment of blood coagulation was performed using Automated Coagulation Timer (ACT) by measuring the clotting time at the three sites investigated (Arm, Passive Leg, Stimulated Leg). A significant drop in clotting time was reported throughout the control and the stimulation study, $p \leq 0.001$; however no significant difference was reported between the sites investigated, $p > 0.05$ refer to **Figure 34** & **Figure 35**. A comparison of the mean (SD) obtained using ACT in both the control and stimulation studies are summarised in **Table 19**.

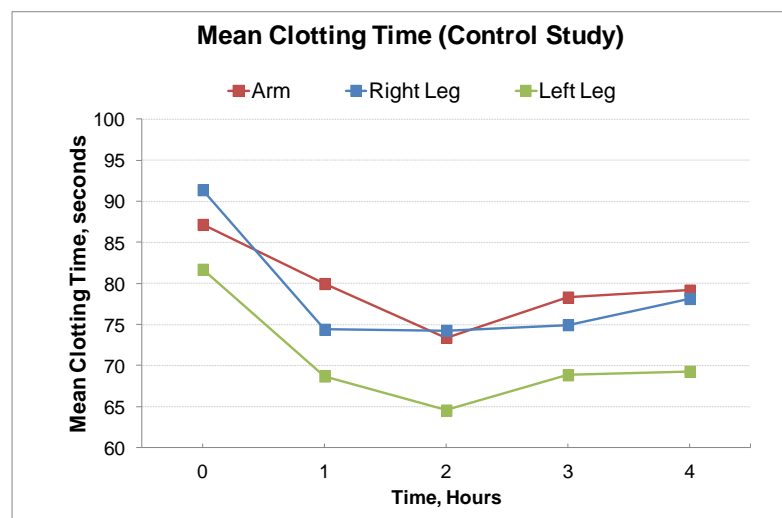


Figure 34: **Mean Clotting Time throughout the Control Study.** Using an Automated Coagulation Timer System (ACT) a significant fall in clotting time was reported at the Arm, Right Leg & Left Leg: $p \leq 0.001$.

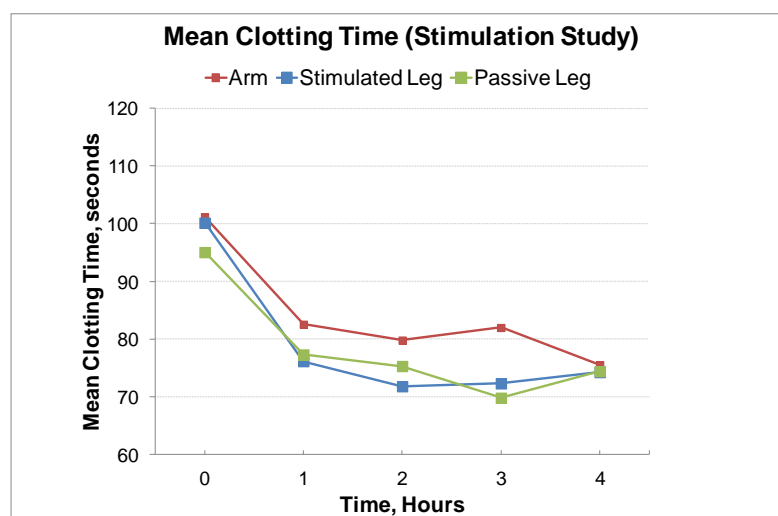


Figure 35: Mean Clotting Time throughout the Stimulation Study. Using an Automated Coagulation Timer System (ACT) a significant fall was reported at the Arm, Stimulated Leg & Passive Leg: $p \leq 0.001$.

Time	Arm Mean (SD)	Right Leg (Stimulated) Mean (SD)	Left Leg (Passive) Mean (SD)
Control Study			
Baseline	87.15 (20.93)	91.38 (14.75)	82.93 (11.49)
1 Hour	79.95 (11.01)	74.38 (8.11)	71.36 (11.96)
2 Hours	73.40 (16.14)	74.25 (11.85)	67.21 (14.07)
3 Hours	78.30 (11.70)	74.94 (9.26)	71.50 (7.99)
4 Hours	79.15 (11.11)	78.13 (10.48)	71.93 (6.04)
Stimulation Study			
Baseline	101.15(10.69)	100.15(12.89)	95.05 (14.24)
1 Hour	82.55 (14.75)	76.15 (14.45)	77.30 (15.35)
2 Hours	79.80 (17.30)	71.80 (10.30)	75.30 (13.49)
3 Hours	83.50 (17.07)	72.30 (13.08)	69.80 (13.88)
4 Hours	77.06 (12.13)	74.25 (12.43)	76.00 (15.05)

Table 19: A Comparison of Clotting Time Measurements obtained using Automated Coagulation Timer System throughout Stimulation and Control Study.

Further analysis of clotting time was performed using Rotem® to confirm the results obtained using the Automated Coagulation Timer System (ACT) in both the control and stimulation study. Since ACT assesses intrinsic coagulation, results were compared to Intem Rotem® results. As with ACT a significant drop in clotting time was obtained in

the stimulation study using Rotem®, $p \leq 0.05$, however all values were in the normal range (100 to 240 seconds) suggesting no abnormal findings, refer to **Figure 36** & **Figure 37**. Likewise, no significant difference between sites was reported, $p > 0.05$. Additional analysis of coagulation parameters using the Intem Rotem device, showed no significant difference in both CFT and MCF across all time points in both the stimulation and control study, $p > 0.05$. Similarly, results were all within the normal range for both CFT (35-110 sec) and MCF (53-72 mm), refer to Appendix 16.

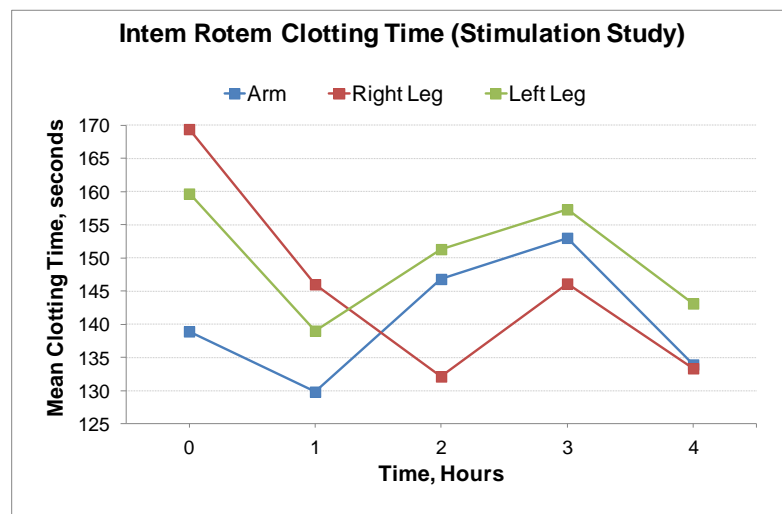


Figure 36: Mean Clotting Time using Intem Rotem® throughout the Stimulation Study. A significant drop in clotting time was reported throughout the study period, $p \leq 0.05$.

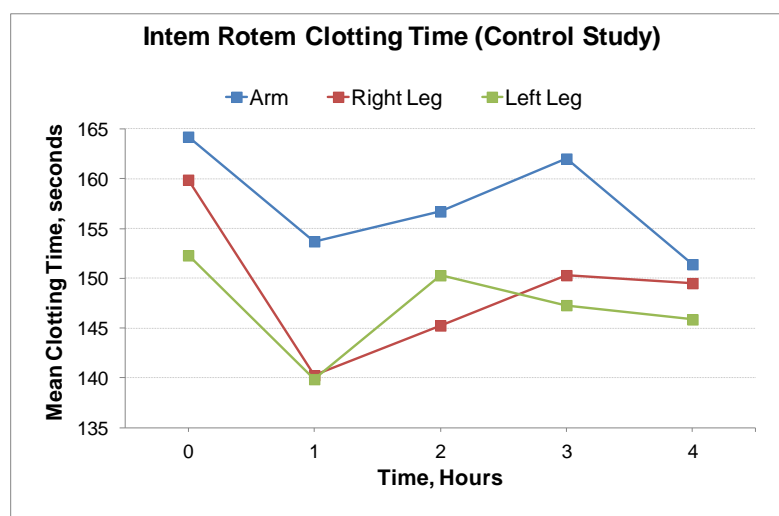


Figure 37: Mean Clotting Time using Intem Rotem® throughout the Control Study. No significant difference in clotting time was reported throughout the study period, $p > 0.05$.

Analysis of the same coagulation parameters using Extem Rotem device, showed a non significant difference in both the stimulation and control study when measuring clotting time and maximum clot formation time, $p > 0.05$ refer to **Figure 38** & **Figure 39**. However, a statistically significant difference among the different sites investigated was reported, $p \leq 0.05$ when measuring clot formation time. Despite that, no significant difference was shown in time throughout the study period, $p > 0.05$ refer to Appendix 16.

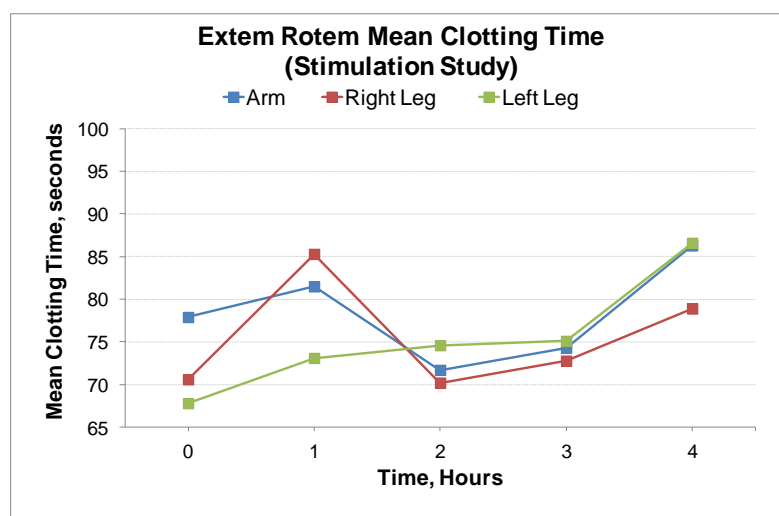


Figure 38: Mean Clotting Time using Extem Rotem® throughout the Stimulation Study. No significant difference was reported throughout the study period, $p > 0.05$.

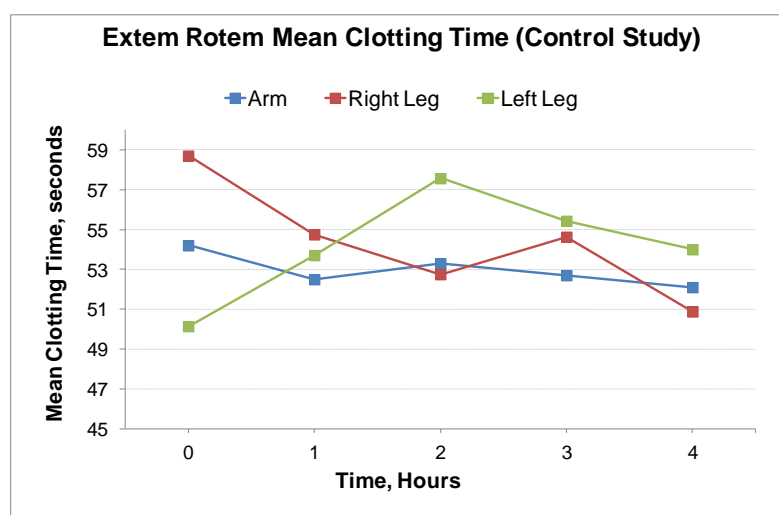


Figure 39: Mean Clotting Time using Extem Rotem® throughout the Control Study, No significant difference was reported throughout the study period, $p > 0.05$.

6.7.4. Assessments of Coagulation Factors

In addition to clotting time, further assessments of specific coagulation factors were performed. These include, measurements of tissue plasminogen activator (tPA) antigen levels, von Willebrand Factor (vWF) antigen, together with 6 keto PGF1 α levels.

Analysis of those parameters was made using Enzyme Linked Immunosorbent Assay (ELISA) technique. Adjusted concentrations were then calculated by deducting the values at baseline from the actual measurements.

6.7.4.1. Assessment of Tissue Plasminogen Activator Antigen

Enzyme Linked Immunosorbent Assay (ELISA) was performed to obtain the concentration of tissue plasminogen antigen in the blood plasma that has been withdrawn at each time point. Tissue plasminogen antigen concentrations were extrapolated using the standard curve plotted following ELISA assay; refer to Appendix 17. Statistical analysis of the results demonstrated a highly significant reduction in tPA antigen levels over time at both the arm and stimulated leg in the stimulation study, $p \leq 0.001$. This is in comparison to a non significant change in the passive leg; $p > 0.05$ refer to **Figure 40**. A comparison of the mean (SD) of both the stimulation and control studies are summarised in **Table 20**. Adjusted percentage change from baseline was then calculated using the actual tPA concentrations, which displayed a fall in tPA antigen levels by 14% in the stimulated leg in comparison to a fall by 10 % at the arm and 1% at the left leg. Further analysis of the tPA antigen data obtained from the control study showed a highly significant drop in the left leg ($p \leq 0.001$) with no significant difference in both the right leg and the arm, $p > 0.05$ refer to **Figure 41**.

Time	Arm Mean (SD)	Right Leg/Stimulated Mean (SD)	Left Leg/Passive Mean (SD)
Control Study			
Baseline	4.29 (4.44)	6.79 (3.92)	8.26 (5.61)
1 Hour	3.54 (3.44)	6.42 (3.93)	7.86 (5.27)
2 Hours	3.48 (2.80)	6.45 (3.61)	6.49 (4.63)
3 Hours	3.87 (3.20)	6.21 (4.32)	6.75 (4.83)
4 Hours	3.33 (3.66)	6.69 (4.10)	6.11 (4.22)
Stimulation Study			
Baseline	7.65 (4.29)	11.43 (8.21)	11.67 (8.53)
1 Hour	6.47 (3.11)	7.47 (3.07)	9.21 (3.64)
2 Hours	6.28 (2.98)	7.13 (2.80)	9.28 (3.73)
3 Hours	5.45 (2.74)	7.19 (2.50)	9.14 (3.29)
4 Hours	5.15 (3.41)	7.49 (3.01)	8.67 (4.58)

Table 20: A comparison of tPA Measurements at the Arm, Right and Left Leg throughout the Stimulation & Control Study.

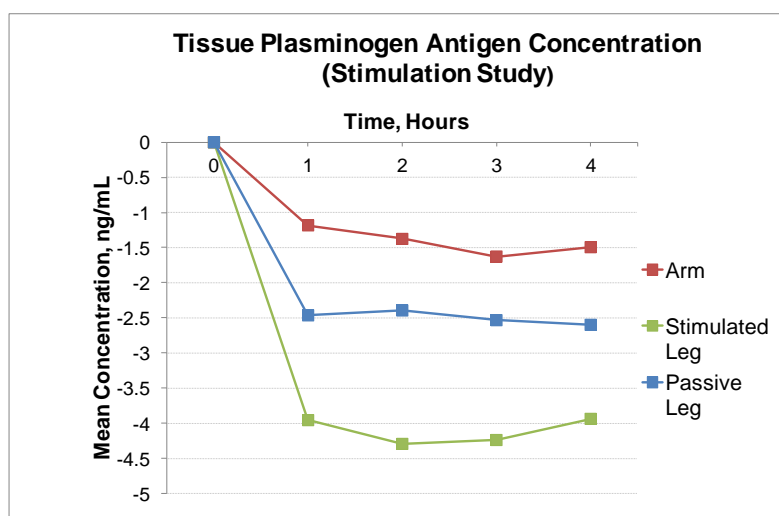


Figure 40: Adjusted Mean tPA Antigen Concentration throughout the Stimulation Study. Arm: $p \leq 0.001$; Stimulated Leg: $p \leq 0.001$, Passive Leg: $p > 0.05$.

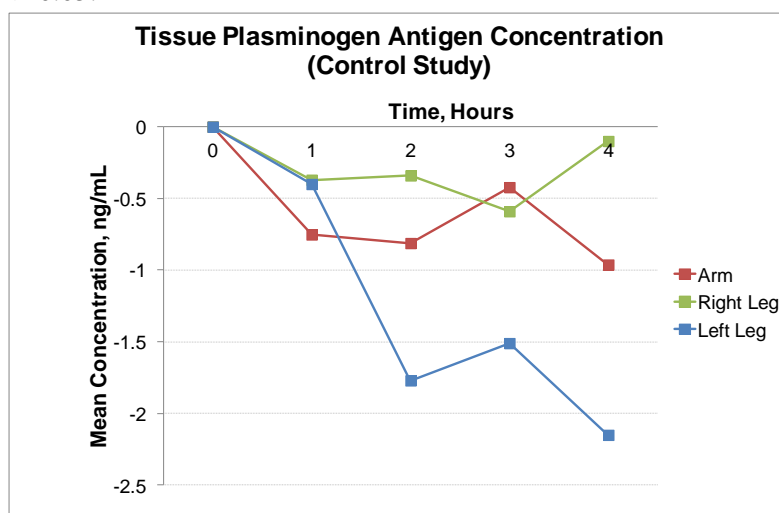


Figure 41: Adjusted Mean tPA antigen Concentration throughout the Control Study. Arm & Right Leg: $p > 0.05$. Left Leg: $p \leq 0.001$.

6.7.4.2. Assessment of von Willebrand Factor

Further assessments of coagulation parameters were performed, by measuring levels of von Willebrand factor (vWF). Enzyme Linked Immunosorbent Assay (ELISA) was performed to obtain the concentration of von willebrand factor in the blood plasma withdrawn at each time point. Concentrations of vWF were extrapolated using the standard curve plotted following ELISA assay, refer to Appendix 18. Statistical analysis

of the data obtained from the ELISA in both the stimulation and control study demonstrated a non significant difference at all sites, $p > 0.05$ refer to **Figure 42** & **Figure 43**. This suggests that electrical stimulation has no effect on vWF synthesis from endothelial cells.

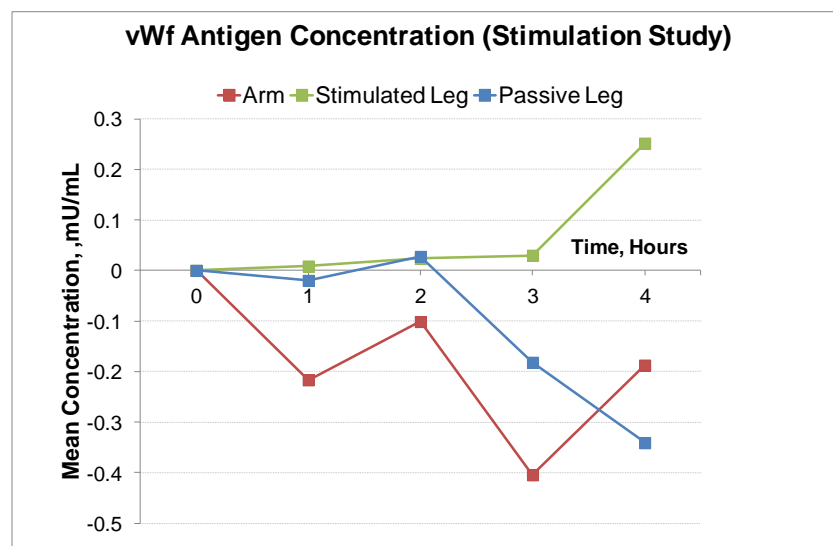


Figure 42: Adjusted Mean vWF antigen Concentration throughout the Stimulation Study, $p > 0.05$.

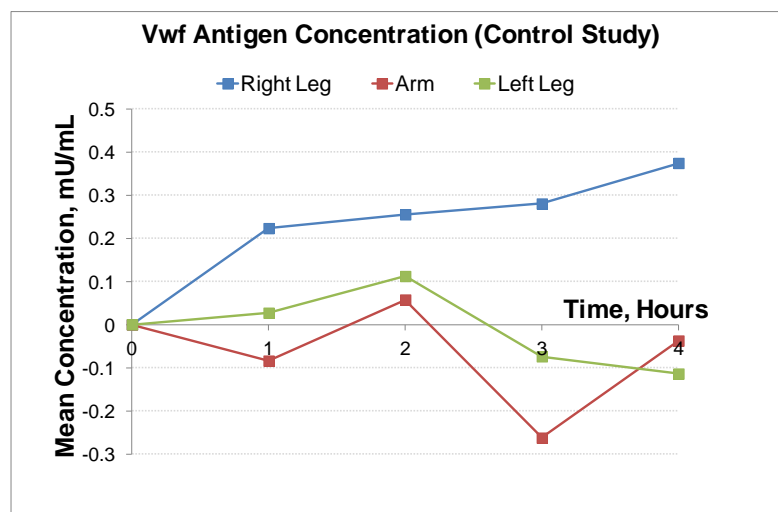


Figure 43: Adjusted Mean vWF antigen Concentration throughout the Control Study, $p > 0.05$.

6.7.4.3. Assessment of 6 Keto PGF1 α

The effect of electrical stimulation on platelet aggregation was investigated through measuring the level of 6 keto PGF1 α , the stable product of prostacyclin. Enzyme Linked Immunosorbent Assay (ELISA) was performed to obtain the concentration of 6 keto PGF1 α in the blood plasma withdrawn at each time point. Concentrations of 6 keto PGF1 α were extrapolated using the standard curve plotted following ELISA assay, refer to Appendix 19. Analysis of the data obtained following the ELISA showed diverse results in both the stimulation and control study. A non significant change was obtained in the levels of 6 keto PGF1 α at all sites throughout the stimulation study; $p > 0.05$ refer to **Figure 44**. This suggests that electrical stimulation displays no direct effect on prostacyclin synthesis. However, further assessments of 6 keto PGF1 α levels throughout the control study were surprising, as a significant change was reported over time, $p \leq 0.05$ refer to **Figure 45**. This was mostly apparent in the left leg, where a highly significant difference was seen over time, $p \leq 0.001$ in comparison to an insignificant difference in both the arm and right leg, $p > 0.05$. Concentrations of 6 keto PGF1 α increased significantly in the left leg with the highest mean value reported at 3 hours (232.64 ng/mL) that was followed by a significant drop to 17.5 ng/mL. This suggests that sitting for a period of 4 hours might have induced platelet aggregation, resulting in the release of prostacyclin from endothelial cells.

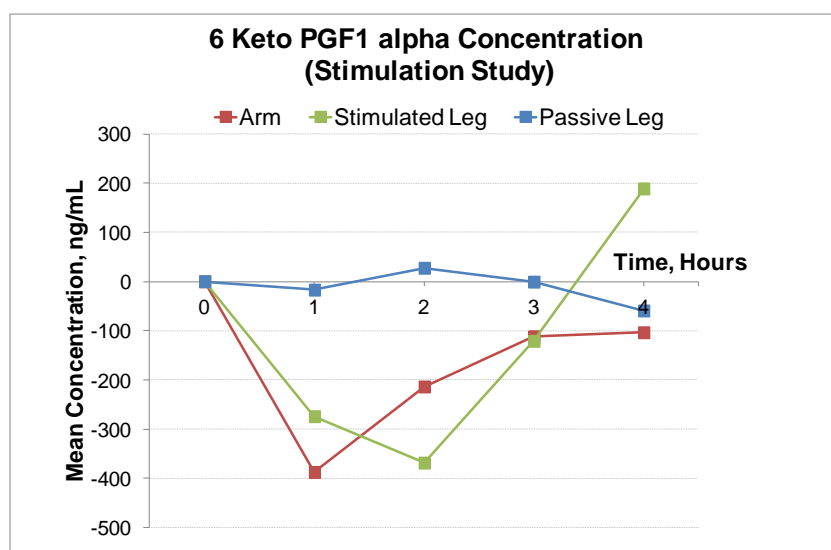


Figure 44: Adjusted Mean Concentration of 6 keto PGF1 α throughout the Stimulation Study. Arm, Stimulated Leg & Passive Leg: $p > 0.05$.

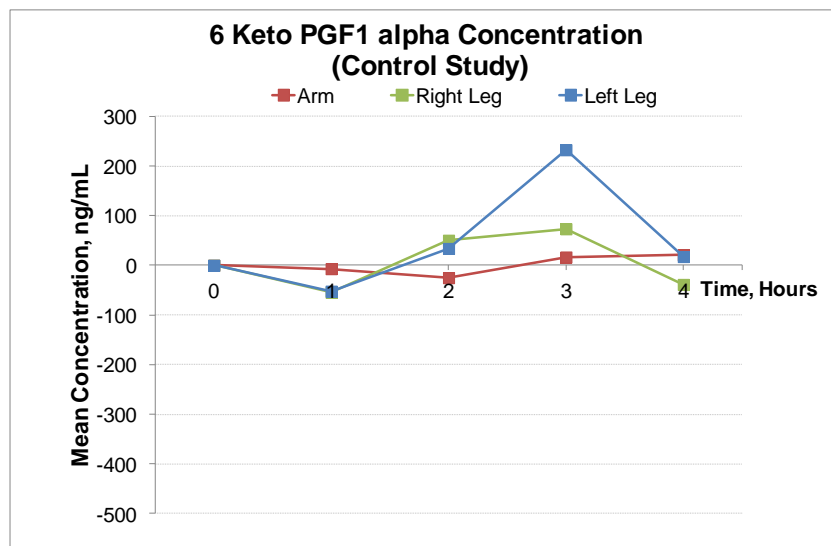


Figure 45: Adjusted Mean Concentration of 6 keto PGF1 α throughout the Control Study. Arm & Right Leg: $p > 0.05$; Left Leg: $p \leq 0.001$.

6.7.5. Assessment of Thrombin Generation & D-dimer Levels

Assessment of the thrombin generating capacity was performed through measuring Lag Time, Endogenous Thrombin Potential (ETP), Peak Height and Time to Peak in blood plasma at each time point. No significant difference in ETP and peak height was reported throughout the different time intervals both in the control and stimulation study, $p > 0.05$ refer to Appendix 20. Interestingly, a significant increase was reported for lag time in the control study as compared to the stimulation study, $p \leq 0.05$. The right leg together with the arm, exhibited a greater lag time in comparison to the left leg, refer to **Figure 46** & **Figure 47**.

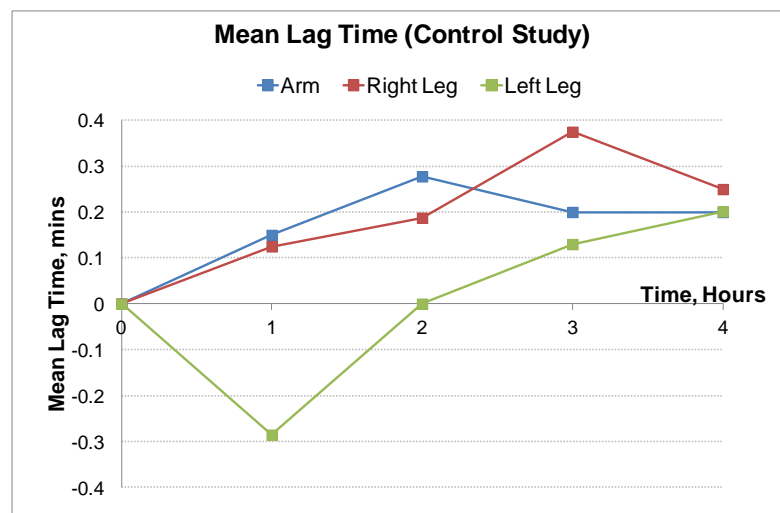


Figure 46: Adjusted Mean Lag Time throughout the Control Study, $p \leq 0.05$.

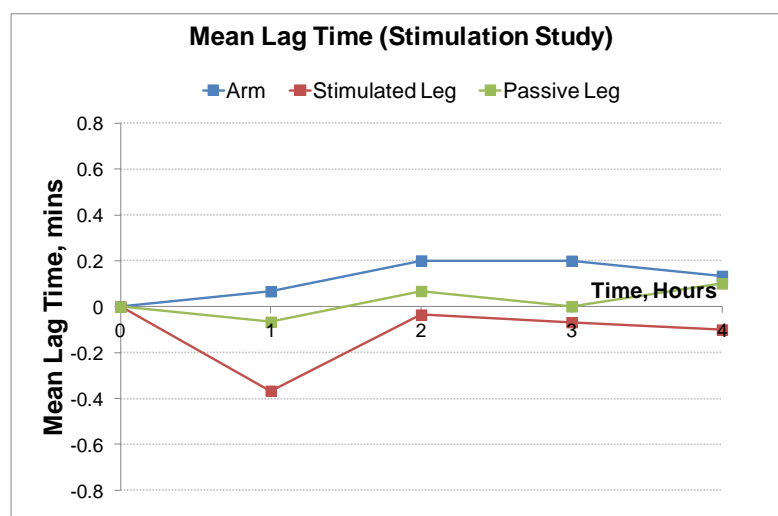


Figure 47: Adjusted Mean Lag Time throughout the Stimulation Study, $p > 0.05$.

Similarly, further data analysis of the control study showed a significant increase in the time to reach the maximum thrombin concentration in comparison to the baseline, $p \leq 0.05$. A gradual increase was observed throughout the study, with the greatest time to peak displayed in the right leg, refer to **Figure 48**.

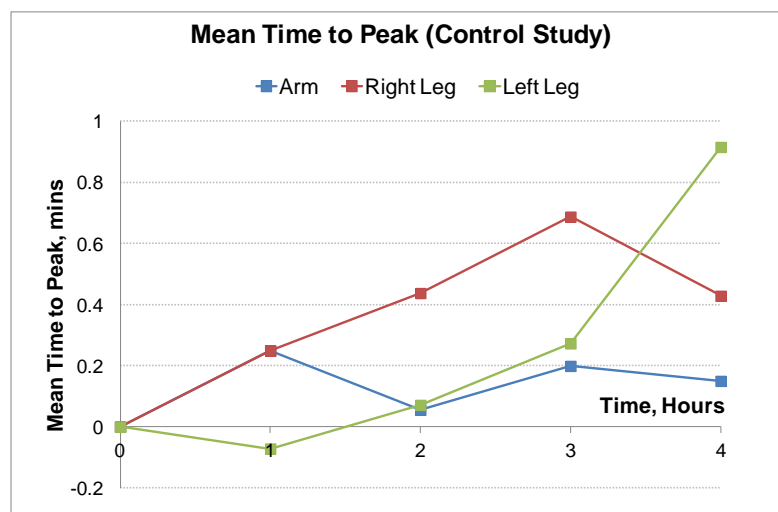


Figure 48: Adjusted Mean Time to Peak throughout the Control Study, $p \leq 0.05$.

Furthermore, assessment of D-dimer levels the final products of fibrinolysis was performed. However, the data obtained from the stimulation study showed similar results at each time point. Based on that, no further statistical analysis was performed and further

assessments of D-dimer levels were not carried out in the control study, refer to Appendix 20 for the D-dimer results obtained.

6.7.6. Safety Assessments

Safety assessments were performed throughout both the control and stimulation study, by measuring oxygen saturation levels, heart rate as well as blood pressure. Data analysis showed no significant difference in oxygen saturation and heart rate compared to the baseline; $p > 0.05$ refer to **Figure 49** & **Figure 50**. Mean oxygen saturation measured using pulse oximetry remained stable and always above 97% throughout the study. Similarly, heart rates remained stable and within the normal healthy range (above 65 bpm) throughout the study.

Interestingly, blood pressure measurements throughout the stimulation study illustrated a statistically significant drop in both systolic and diastolic pressure, $p \leq 0.001$ refer to **Figure 51**; this is in comparison to a non significant change in the control study refer to **Figure 52**. A comparison of the mean (SD) values reported in both the control and stimulation study are summarised in **Table 21**.

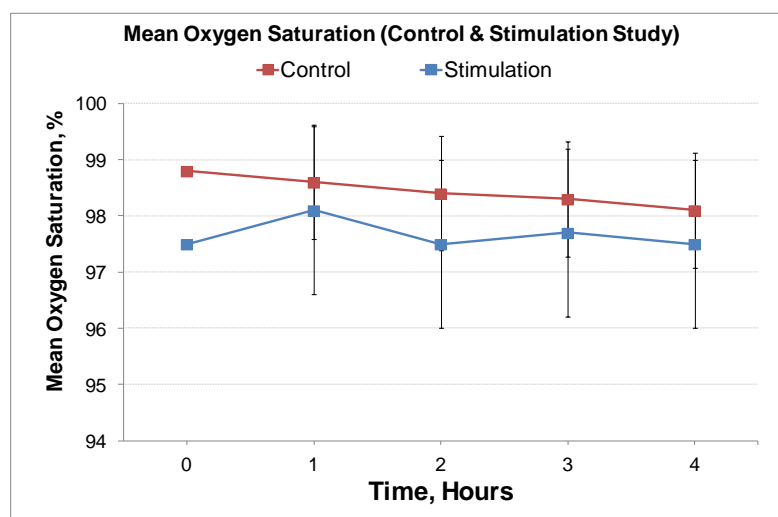


Figure 49: Mean Oxygen Saturation Levels throughout the Control & Stimulation Study, $p > 0.05$. Error bars represent 95% confidence interval of the difference from baseline. Oxygen levels remained stable and above 97% throughout both the control and stimulation studies.

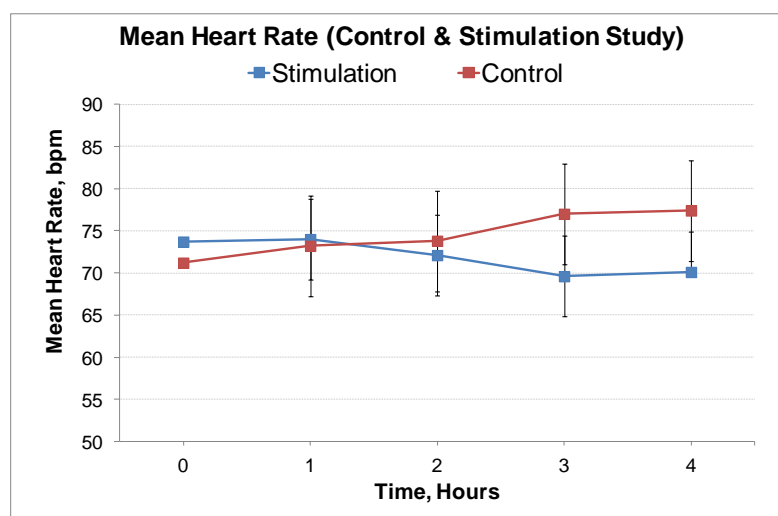


Figure 50: Mean Heart Rate throughout the Control & Stimulation Study, $p > 0.05$. Error bars represent 95% confidence interval of the difference from baseline. Heart rate remained stable and within the normal range throughout both the control and stimulation study.

Time	Systolic Blood Pressure Mean (SD)	Diastolic Blood Pressure Mean (SD)
<i>Stimulation Study</i>		
Baseline	131.60 (14)	83.10 (11.75)
1 Hour	129.10 (17)	79.60 (14.25)
2 Hours	124.50 (16.21)	75.60 (14.84)
3 Hours	125.40 (16.56)	74.40 (14.28)
4 Hours	126.10 (11.08)	72.30 (11.83)
<i>Control Study</i>		
Baseline	127.70 (13.90)	76.80 (8.50)
1 Hour	125.20 (11.10)	79.10 (15.01)
2 Hours	119.80 (18.75)	74.10 (12.40)
3 Hours	126 (10.18)	78.30 (15.33)
4 Hours	125 (12.54)	73.60 (8.76)

Table 21: A comparison of Mean (SD) Blood Pressure Measurements throughout the Stimulation & Control Study

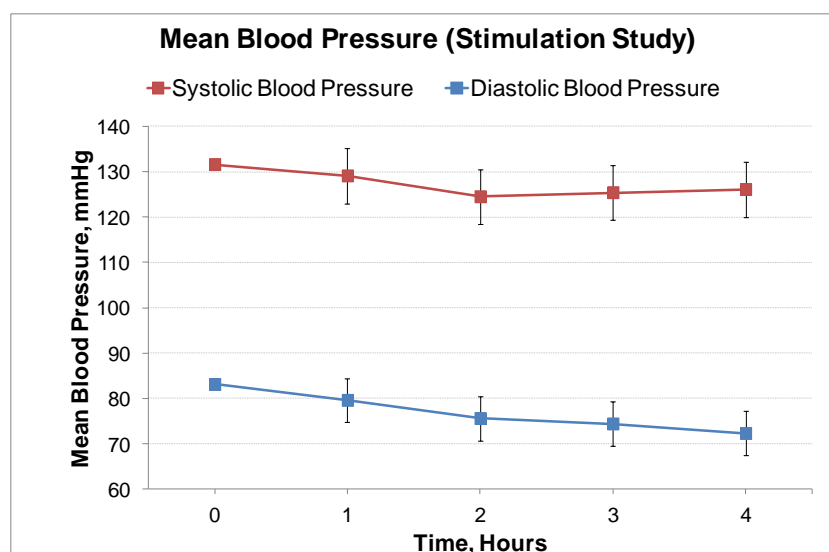


Figure 51: Mean Blood Pressure Measurements throughout the Stimulation Study, $p \leq 0.001$. Error bars represent 95% confidence interval of the difference from baseline. A small but a significant drop in both systolic and diastolic blood pressure was reported throughout the study period.

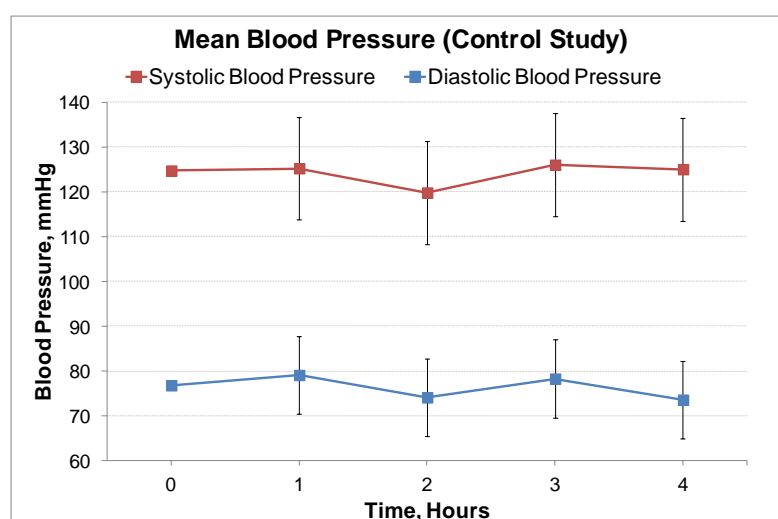


Figure 52: Mean Blood Pressure Measurements throughout the Control Study, $p > 0.05$. Systolic and diastolic blood pressure remained stable and within a normal range throughout the study period.

6.7.7. Discomfort Assessments

Analysis of the discomfort levels showed no significant difference following stimulation at each time point, $p > 0.05$. Using the Verbal Rating Score (VRS), the majority of volunteers reported mild discomfort for the electrical nerve stimulation over time, as

characterised by a mean score of 2.6 out of 5 at all time points, refer to **Figure 53**. Moreover, electrical nerve stimulation was rated at minimal sensation, as the mean score reported at all time points was 35.8 out of 100 using the visual analogue score (VAS), refer to **Figure 54**.

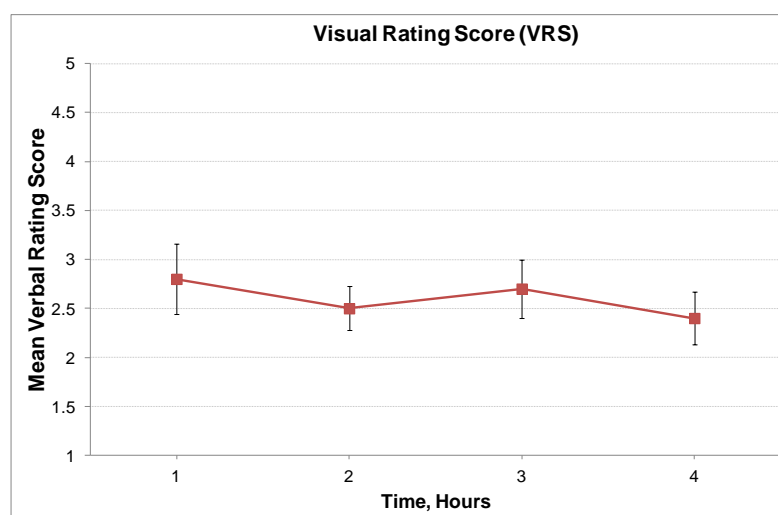


Figure 53: Mean Visual Rating Score throughout Stimulation, $p > 0.05$. Discomfort pain was rated on a scaled between 1-5; 1 = no sensation (other than muscle tensing and relaxing and 5 = severe discomfort. A mild discomfort was perceived following stimulation at all time points

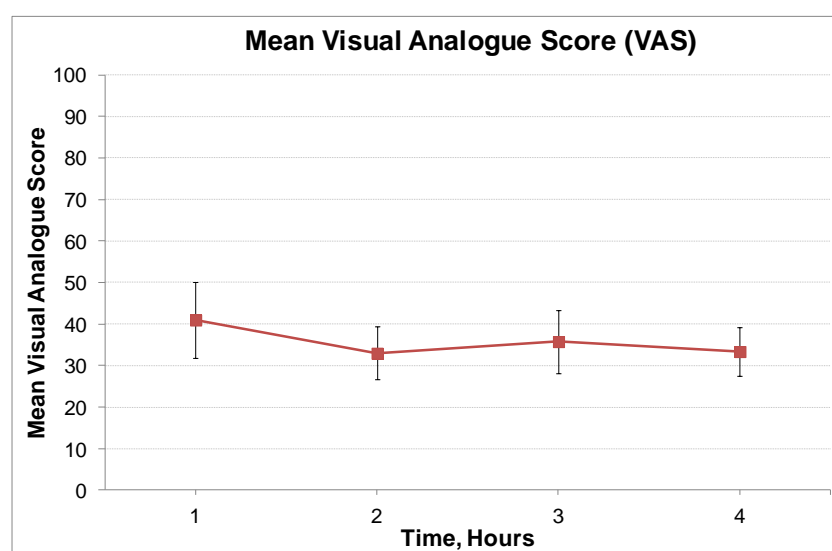


Figure 54: Mean Visual Analogue Score throughout Stimulation, $p > 0.05$. The level of pain was marked using a 100mm line marked at one end 'no sensation' and at the other end 'severe discomfort.' A minimal sensation was perceived using the stimulation at all time points.

6.8. Discussion

A number of commercially available mechanical prophylaxis devices exist however, they are associated with several drawbacks such as being uncomfortable and difficult to apply or use, cumbersome and restrict mobility. In the quest for alternative methods for thromboembolic prevention, electrical stimulation stands out as a method producing encouraging results for a variety of conditions especially the prevention of deep vein thrombosis (Velmahos et al., 2005). Its effectiveness in reducing the incidence of preoperative and postoperative DVT has been reported widely (Browse and Negus, 1970, Doran et al., 1970, Lindstrom et al., 1982, Nicolaides et al., 1972, Velmahos et al., 2005). However, as direct electrical stimulation is extremely painful, this method has only been applied in restricted situations, i.e. in a controlled hospital environment under general anaesthesia.

Preliminary studies were carried out to examine the effectiveness of indirect electrical stimulation via the popliteal nerve and the resulting activation of venous muscle pumps of the lower leg (Tucker et al., 2010). Results obtained demonstrated that the novel electrical stimulation technique is safe, and significantly increases blood flow and velocity by magnitudes that have not been shown previously. Following the significant results obtained, the present study was carried out to further evaluate the efficacy of the novel electrical nerve stimulation technique from multiple perspectives. The effect of electrical stimulation on peripheral circulation, vital signs and multiple blood coagulation parameters was investigated.

6.8.1. Electrical Stimulation Effect on Peripheral Circulation

Adequate peripheral blood flow in the lower limb is essential to maintain haemostatic balance and prevent venous stasis. Colour flow duplex ultrasound assessments to the arterial and venous femoral blood vessel at different time points (Baseline, 1, 2, 3, and 4 hours) were performed during the stimulation and control study. A significant increase in velocity, as well as blood volume flow was reported.

To our knowledge, the technology studied is novel and to date none of the studies carried out on electrical stimulation devices used a similar mechanism of action. Therefore, comparison of the results will be based on similar parameters measured by different electrical stimulation techniques. For instance, although the magnitude of increase is different, the results obtained are in agreement with previous studies carried out by Kaplan et al, using direct muscle stimulation, as a significant increase in venous velocity was reported. (Kaplan et al., 2002). The increase in venous velocity has also been demonstrated in another study using foot impulse technology, where the peak venous femoral velocity reported was 59.79 cm/sec following the use of an inflation device in comparison to a baseline value of 13.25 cm/sec. Others studies have also shown a significant increase in peak venous velocity that ranged between 43 to 120 cm/sec when measured in the popliteal vein using a muscle stimulation device at a high frequency (50Hz) (Izumi et al., 2010). Thus, as with the previous studies, the results reported in our study demonstrates a significant increase in venous velocity measurements. The greatest peak venous velocity measurement obtained was 30 cm/sec that was reached at 3 hours, which is lower than that reported by the latter studies. However, it is important to consider the different mechanism of action and the different setting modes used. The THRIVE device used in this study stimulated the peroneal nerve. It was applied for 5 minutes every 15 minutes period and the electrical stimulation displayed is constant and pulsatile with a very low frequency (3 Hz).

Furthermore, a highly significant increase in blood volume flow was demonstrated in the femoral vein, $p \leq 0.001$. The increase reported in venous volume flow is equivalent to that reported by other electrical stimulation studies. Following a baseline volume flow of 59.4 mL/min the greatest volume was again reported at 3 hours were a volume flow of 253.6 mL /min was obtained. This is similar to the increase reported at the popliteal vein (258mL/min) by (Izumi et al., 2010) and higher than the increase reported by (Griffin et al., 2010) (240mL/min) following electrical stimulation. Moreover, the average percentage increase from baseline demonstrated in this study, which ranges between 405% and 489% is greater than that shown in another study using neuromuscular electrical stimulation (NMES) where the highest mean average change reported was 301%

following a 4 hour stimulation period (Broderick et al., 2010a). In addition to the increase in venous volume flow, a significant increase in arterial volume flow was reported following stimulation, this is compared to a significant fall reported in the control study, $p \leq 0.05$. As with previous measurements, following stimulation, the highest increase in volume flow was reported at 3 hours where the blood volume obtained was 259.4 mL/min. On the contrary, arterial volume flow decreased from 124.99 mL/min at baseline to 111.2 mL/min at 4 hours in the control study. This suggests a possible systemic effect of the stimulation device. As studies investigating electrical stimulation techniques are mostly concerned with the effect on venous blood vessel and do not measure arterial measurements, the arterial results obtained in the present study cannot be compared with others.

The enhanced effect of electrical stimulation was also apparent when measuring skin perfusion bilaterally in the dorsum of the feet using laser Doppler flowmetry (LDF). The main findings of the present study are a statistically significant increase in LDF in the stimulated leg as compared to the passive leg in the stimulation study, as compared to an insignificant difference in the control study. The skin perfusion reported at baseline was 4.66 flux units at baseline, which escalated to 73.59 flux units following 1 hour and continued to increase reaching 75.85 flux units at 4 hours. The results obtained in this study further confirm the enhanced effect of the electrical stimulation on the skin microcirculatory velocity as reported earlier in an preliminary study carried out by (Tucker et al., 2010).

6.8.2. Electrical Stimulation Effect on Blood Coagulation Parameters

The effect of the novel electrical stimulation on specific coagulation factors was investigated by measuring tissue plasminogen activator (tPA) antigen, von Willebrand Factor (vWF) and Prostaglandin PGF1 alpha throughout the study period. The systemic effects of electrical stimulation was reported earlier by (Nicolaidis et al., 1972), where muscle contractions was shown to increase the fibrinolytic activity in the blood. Levels of tissue plasminogen activator antigen were significantly reduced throughout the stimulation and control study. However, the reduction level was greater in the stimulation

study than the control. Following stimulation, a highly significant reduction in tPA antigen levels was demonstrated in the stimulated leg and the arm ($p \leq 0.001$). Tissue plasminogen activator antigen levels reflect both tPA and tPA bound to PAI-1 and do not represent tPA levels alone; also most of the circulating tPA is bound to PAI-1 (Comerota et al., 1997a, Nordenhem and Wiman, 1998). A direct relationship exists between tPA antigen and PAI-1 levels. As mentioned earlier, a decreased fibrinolytic activity is associated with increased levels of PAI-1 hence, increased levels of tPA. Therefore, the drop in tPA concentration in the plasma might suggest an increased fibrinolytic activity, which may also be correlated with a drop in PAI-1. The increased fibrinolytic activity is beneficial to break down any clots formed. To confirm this, further assessments must be performed by measuring PAI-I levels. However, plasma samples were not sufficient to perform this. Many prospective studies have been published, indicating that a decreased fibrinolytic activity due to increased concentrations of tPA antigen and PAI-1 are predictors of myocardial infarction (Held et al., 1997, Juhan-Vague et al., 1996, Nordenham et al., 2005, Scarabin et al., 1998, Thogersen et al., 1998). This suggests that the drop in tPA antigen demonstrated by the electrical stimulation might be of beneficial use for cardiovascular patients. However, further tests are needed to confirm this.

Interestingly, no significant change in plasma vWF was seen throughout the stimulation and control study, suggesting a minimal effect of electrical stimulation on endothelial cell activation. Raised levels of plasma vWF antigen is an indication of endothelial cell activation and dysfunction following venous occlusion (Scharrer and Vigh, 1993). Other earlier studies have also demonstrated that increased vWF level represent a risk factor for DVT (Nilsson et al., 1986, Stead et al., 1983). However, these findings were challenged by a later study, where it has been found that vWF did not differ in patients with DVT versus healthy subjects (Koster et al., 1995). Thus, the non significant change in vWF is justified.

Furthermore, prostacyclin was also assessed throughout the study by measuring its stable marker 6 keto prostaglandin F1 α . Following stimulation, a non-significant change in plasma levels of 6 keto prostaglandin F1 α was observed at all sites. This suggests that the electrical stimulation has no direct effect on the arachidonic acid metabolism that results

in prostacyclin synthesis. However, analysis of 6 keto prostaglandin F1 α throughout the control study showed conflicting results, as a significant increase followed by a gradual drop in prostacyclin levels in the left leg. A possible explanation to the increase in prostacyclin shown, is that the sitting induced platelet aggregation, which has resulted in the release of prostacyclin to regulate the aggregation process. Following regulation, levels of prostacyclin then dropped at 4 hours.

The effect of the novel electrical stimulation technique on blood coagulation was further investigated by assessing other coagulation parameters. For instance, clotting time was measured using two techniques, ACT and Rotem®. Contrary to our expectations, in both the stimulation and the control study a statistically significant drop in blood clotting times was demonstrated throughout the study period using the ACT technique. The results were further confirmed following the use of the Rotem® device, which unlike ACT, is a more sensitive technique that provides a complete evaluation of the process of clot initiation and structural characteristics of the clot formed and its stability (Mallett and Cox, 1992). A statistically significant drop in clotting time was demonstrated in the stimulation study using Rotem®. This suggests that there may be a systemic increase in coagulation taking place during the study period. Despite that, all values reported using both techniques were within the normal range.

Further assessment of blood coagulation was performed by measuring the endogenous thrombin potential (ETP), which is one of the main determinants of haemostasis and thrombosis (Vlieg et al., 2007). An elevated level of ETP is usually associated with an increased risk for DVT (Eichinger et al., 2008, Vlieg et al., 2007). Assessment of other secondary components of the thrombin generation was also performed by measuring the peak height, lag time, and time to peak. However, according to Vlieg et al, none of those components are associated with an increased risk for DVT (Vlieg et al., 2007). Results obtained in this study showed no significant change in ETP levels together with peak height throughout the study period. Interestingly, a significant increase in lag time and time to peak was demonstrated throughout the study. This suggests that coagulation is taking place, however since other measurement techniques showed opposite results, it is difficult to confirm this. It must be remembered that this study has been conducted on

healthy volunteers and the sample number is small. So a better way of confirming the findings obtained, is to repeat the study on a greater patient population.

6.8.3. Electrical Stimulation Effect on Vital Signs

Safety of the novel electrical stimulation device was of prime concern in this study. The safety of the THRIVE device was evaluated by monitoring oxygen saturation (SPO₂) levels, heart rate as well as blood pressure throughout the study. The majority of the studies published earlier report only the change in the heart rate following electrical stimulation and the reported data are conflicting. A lower heart rate was demonstrated when subjects lying supine were exposed to electrical stimulation (Faghri et al., 1997). On the other hand, an increase in heart rate was noted when subjects lying supine were exposed to another novel electrical stimulation device (Broderick et al., 2010b) suggesting discomfort. A non-significant change in mean oxygen saturation and heart rate was observed throughout both the stimulation and control studies. Both oxygen saturation and heart rate values were stable and within the normal range. Since, measuring vital signs using pulse oximetry requires adequate peripheral blood flow; the enhanced blood flow evoked by electrical stimulation was beneficial for the measurement of the vital signs.

Unlike oxygen saturation and heart rate results, a small but statistically significant drop in both the systolic and diastolic blood pressure was demonstrated throughout the stimulation study. The systolic pressure dropped from 131 mmHg at baseline to 126 mmHg at 4 hours. Similarly, the diastolic pressure dropped was 83 mmHg at baseline to 72.3 mmHg at 4 hours. The drop demonstrated is not of clinical significance, as results were within the normal range. The fall of blood pressure could be due to the volunteer being seated for a prolonged period of time and hardly consuming a significant quantity of food. Such results could imply that the device may be of importance to hypertensive patients. However further investigations are needed to confirm this.

6.8.4. Tolerance and Acceptance to Electrical Stimulation

Given the wide range of side effects associated with the currently available thromboprophylaxis measures. Levels of discomfort was assessed following stimulation using the verbal rating score (VRS) and visual analogue scale (VAS). Subjects reported a mean rating score of 3 out of 5 using the verbal rating score, suggestive of mild discomfort sensations. Similarly, a mean score of 35.8 out of a 100 was reported using the visual analogue scale, suggesting a minimal sensation. The mild discomfort perceived following exposure to the novel electrical stimulation device, is similar to that reported by (Broderick et al., 2010b) following 4 hours of electrical stimulation.

Results of this study, suggests further that contractions of the calf and foot pumps evoked by the novel electrical stimulation device are effective in improving lower limb perfusion at the vascular and micro-vascular level. The resulting effect is an improvement in venous return and prevention of venous stasis, thereby potentially preventing deep vein thrombosis. The device also enhances fibrinolytic activity, hence influencing blood coagulability. Thus, the electrical stimulation used targets two components of the Virchow's triad; venous stasis and blood coagulability. Also, the electrical stimulation device has no profound effects on vital signs. It is well tolerated by healthy subjects as it is only associated with mild discomfort. It is clear from this study and previous studies carried out, that electrical stimulation is an effective thromboprophylaxis technique that might be useful in hospital settings. To prove this, further investigations must be carried out on patients to evaluate its clinical effectiveness. However, prior to initiating the trials on patients, it was of interest to evaluate how the device performs in relation to commonly used devices in hospital settings. This will further provide an idea of the potential usage of the novel device investigated.

The two most commonly used prophylaxis methods in hospitals are the Graduated Compression Stockings (GCS) and Intermittent Pneumatic Compression (IPC) devices. Since the effectiveness of the GCS in the prevention of DVT was questionable (CLOTS, 2009), IPC devices were selected for the comparison. Further research was therefore performed comparing the effectiveness of the novel device with the IPC devices.

Chapter 7. The Effectiveness of the geko™ Medical Device versus Intermittent Pneumatic Compression: A Comparative Study

7.1. Introduction

Thromboprophylaxis measures fall into two categories, pharmacological and mechanical methods. Numerous studies validated the efficacy of pharmacological agents (Francis et al., 1997, Hull et al., 1979, Lensing et al., 1995, Lotke et al., 1996, Markel and Morris, 2002). Despite that, the bleeding risk and the need of clinical supervision remains a major challenge (Cohen et al., 2007, NHS, 2012, Qaseem et al., 2011, Welch, 2010). In recent years, mechanical prophylaxis for the prevention of deep vein thrombosis (DVT) has enjoyed wide popularity, as its use is not associated with the adverse events seen with pharmacological prophylaxis (Morris and Woodcock, 2004).

Intermittent pneumatic compression (IPC) devices are one of the most commonly used mechanical prophylaxis methods that are widely used both intra-operatively and post operatively to prevent DVT (Izumi et al., 2010). All IPC devices have the same general principle, to compress the limb, expel blood from the underlying superficial and deep veins, which if the valves are competent will be displaced proximally (Morris and Woodcock, 2004). Compression is achieved through a pumping action that inflates and deflates air bladders within cuffs that can cover the whole leg, the calf, or just the feet. The deflation action is responsible for the refill of the veins ensuring the periodic flow of the blood through the deep veins, hence preventing venous stasis. The major disadvantage associated with the IPC devices is their size, weight, and requirement for continuous attachment to a power supply that contribute to poor patient compliance. Patient compliance has been argued as one of the most important factors to consider when selecting a mechanical prophylactic device (Morris and Woodcock, 2004).

Alternative prophylaxis measures for the prevention of DVT include direct electrical stimulation of the lower limb muscles, which proved to be an effective modality in

improving blood flow (Currier et al., 1986, Faghri et al., 1997, Kaplan et al., 2002). However, the elevated level of discomfort associated with their use at high intensities limited its practical use to anaesthetised patients only.

Recently a novel electrical stimulation device (geko™ T-1) has been developed by Firstkind Ltd, High Wycombe UK. The system operates using OnPulse™ Technology, activating foot and calf pumps of the leg by low intensity neuromuscular electrical nerve stimulation of the common peroneal nerve located within the popliteal fossa (Tucker et al., 2010). The geko™ T-1 device has been CE marked and its intended use is to increase blood circulation for the prevention of venous thrombosis. It can generate 7 pulse width settings ranging from 70µs to 560µs. The desired pulse width can be selected by an on-off switch button.

7.2. Objectives

The main objective of the present study was to compare the effectiveness of a novel medical device, geko™ T-1(Firstkind Ltd, UK) at low and higher pulse widths in enhancing lower limb blood perfusion with two leading IPC devices, Huntleigh Flowtron Universal™ (Huntleigh Healthcare Ltd, UK) and Kendall SCD Express™(Covidien plc, Ireland). Furthermore, subjects' tolerance and acceptability to the devices will be compared using a discomfort questionnaire.

7.3. Study Approval

The study was approved by the North London Research Ethics Committee 1 (reference 05/Q0408/14), on 7th January 2011, refer to Appendix 21.

7.4. Materials & Methods

7.4.1. Volunteers

Ten healthy volunteers (8 Males, 2 Females), were recruited by advertisement to staff and students at Barts and the London, Queen Mary University, Barts and The London NHS

Trust and to the general community refer to **Table 22**. The specific inclusion and exclusion criteria for volunteer selection are presented in **Table 23** & **Table 24**. Volunteers were instructed to have a light breakfast and avoid fatty foods and caffeine containing products. All volunteers attended The Ernest Cooke Clinical Microvascular unit at St Bartholomew's Hospital where assessments were performed. Volunteers were provided with information sheets, and written informed consent for screening and study participation was obtained prior to the study refer to Appendix 22 & 23.

Parameters	Mean (SD)	Median [Range]
Sex; M:F	8:2	
Age; years	39 (8.12)	40.5 [29- 50]
Body Mass Index (BMI)	24.8(2.44)	24.8 [19.9 – 28.8]

Table 22: Demographic Data for geko™ vs. IPC Comparison Study.

7.4.2. Study Procedure

7.4.2.1. Screening Evaluation

After explaining the study procedure and following obtaining informed consent, volunteers were screened prior to starting the study. The screening evaluation included a medical history, physical examination together with vital signs (blood pressure and heart rate). Ultrasound assessment to the lower limb was also performed, examining the perforators, deep and superficial venous systems to exclude the presence of thrombus.

Health	Good General Health
Age	Between 18 and 65 years
Medical History	No abnormal findings; absence of DVT and haematological disorders;
Body Mass Index (BMI)	Between 18kg/m ² and 34kg/m ²
Ankle Brachial Pressure Index (ABPI)	Normal ABPI > 0.9
Drugs	No history of drug abuse (including alcohol)
Medication	No medication during 30 days preceding or during the study

Table 23: Inclusion Criteria for geko™ T-1 vs. IPC Comparison Study.

Health	Organ dysfunction, any clinically significant deviation from normal in the physical determinations
Age	<18 or >65 years
Medical History	Haematological disorders, previous DVT/PE, varicose veins or lower limb ulceration, musculoskeletal disorders, recent surgery and recent trauma to lower limb; and history of gastrointestinal, hepatic, renal, cardiovascular, endocrine, neurological, dermatological, rheumatological, metabolic (including diabetes), psychiatric, or systemic disease judged to be significant
Body Mass Index (BMI)	Chronic obesity (BMI >34 kg/m ²)
Ankle Brachial Pressure Index (ABPI)	Peripheral arterial disease (ABPI <0.9)
Medication	Any medication in the previous 30 days
Participation in other clinical study	Participation in any clinical study during the 8 weeks preceding the active period of the study

Table 24: Exclusion Criteria for geko™ T-1 vs. IPC Comparison Study.

7.4.3. Study Methodology

The study was performed in a room where the temperature and humidity was controlled ($24 \pm 1^{\circ}\text{C}$, relative humidity 30-40%). Volunteers clad in shorts, lay supine on a padded table that could be tilted manually, with their heads supported by a pillow and tilted upwards to 45° . After 30 minutes of supine rest, baseline measurements were recorded. Devices to be tested were then fitted bilaterally to the subject's limbs in accordance with the manufacturer's instructions, in a sequential manner.

The order of the device tested was made in accordance to a pre-set randomisation schedule refer to Appendix 24. Each device was active for a period of 30 minutes followed by 10 minutes recovery phase, to allow vascular re-equilibration prior to switching on the next device. A series of non-invasive measurements were performed at baseline and at the end of each programme. Changes in blood flow and volume, together with microcirculatory velocity were measured using laser Doppler flowmetry (Laser

Doppler Perfusion & Temperature Monitor DRT4; Moor Instruments Ltd, UK) and colour flow duplex ultrasound (Philips IU22; Philips Healthcare, USA).

Several safety assessments were also performed, these included measuring blood pressure using a digital blood pressure monitor (UA-767PC; A&D Instruments Ltd, UK), pulse oximetry (3900 TruTrack®+ Datex-Ohmeda Ltd, UK) together with transcutaneous tissue oxygen tension (TCM4 Tina; Radiometer Ltd, UK). Transcutaneous tissue oxygen tension is a non-invasive measurement for assessing the peripheral circulation (Byrne et al., 1984). It reflects the amount of oxygen that diffuses from the capillaries, through the epidermis to an electrode placed at the site of measurement. The general principle for the technique involves the application of heat to the skin, producing hyperaemia, which enhances blood perfusion thereby increasing oxygen pressure. Following calibration of the electrodes in buffer solution, the electrodes were attached bilaterally on the dorsum of the feet by an adhesive ring. Oxygen diffusion was then measured and displayed on a monitor as the transcutaneous oxygen pressure, $TcPO_2$.

7.4.3.1. Electrical Nerve Stimulation using the geko™T-1 Device

Transcutaneous electrical nerve stimulation was performed using a novel device (geko™ T-1, FirstKind Ltd, UK), refer to **Figure 55**. The geko™ T-1 device is a small, disposable, internally powered, self adhesive device that is applied over the common peroneal nerve (also called the lateral or medial popliteal nerves) located within the popliteal fossa behind the knee. The specifications of the geko™ device are listed in **Table 25**. Stimulation of these nerves by the geko™ T-1 device result in isometric contraction of the muscles that enhance blood flow from the lower limbs back to the heart, thereby increasing venous return (Tucker et al., 2010). The device has 7 stimulation settings relating to pulse width ranging from 70 to 560µs, set by the on-off switch and indicated by a flashing light (setting 1 = lowest; setting 7 highest). Unlike many electrical stimulation devices, the device operates at a fixed frequency (1Hz) with a constant pulse current of 27mA. The geko™ T-1 was applied bilaterally to each subject according to the instruction manual supplied refer to Appendix 25. For each subject, the device was initially switched on at a low setting for a period of 30 minute followed by a 10 minute rest. A low setting was defined as the lowest possible stimulation level that

results in a minor visible twitching movement of the calf and foot. Following the 10 minute resting period, a higher pulse width was selected that was set to 3 additional levels to the previous low setting.

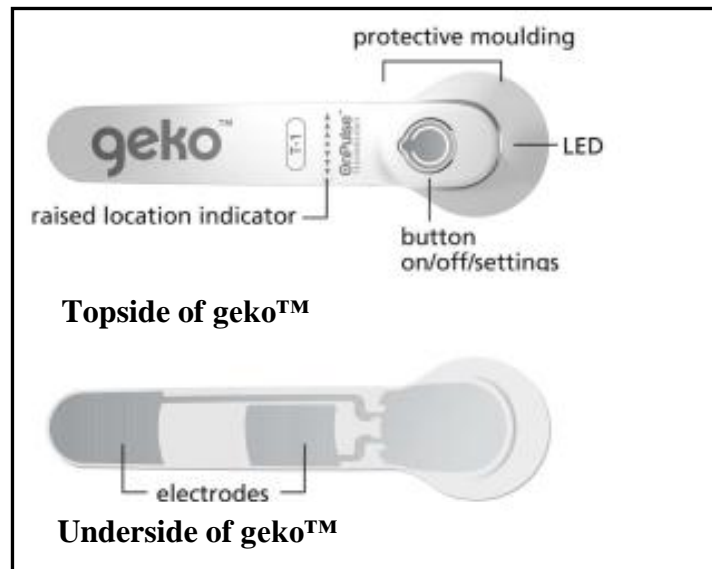


Figure 55: The geko™ T-1 Device.

7.4.3.2. Intermittent Pneumatic Compression (IPC)

The two intermittent pneumatic compression devices used in this study were Huntleigh Flowtron Universal™ (IPCHF) and Kendall SCD Express™ (IPCKendall). Each device was applied bilaterally to the calf as per the manufacturer's instructions; refer to Appendix 26 & 27. The devices differ in their pumping cycle. The IPCHF device has a pumping cycle characterised by 13 seconds of inflation period and 47 seconds deflation period. In contrast, the IPCKendall device has approximately 12 seconds inflation and 48 seconds deflation period; the periodicity of the cycling of this device is influenced by the venous refilling times. The compression pressure was kept the same for both IPC devices (40 mmHg).

Weight	18g
Dimensions	149mm x 42mm x 11mm
Stimulation Mode	7 selectable pulse width
Pulse Width	70,100,140,200,280,400,560 µs
Pulse Current	27 mA (± 15%) constant current
Repetition Rate	1 Hz (± 5%)

Table 25: Specifications of the geko™ T-1 Device.

7.4.3.3. Ultrasound Assessments

Ultrasound assessments were performed using colour flow duplex ultrasound (Philips IU22; Philips Healthcare, USA) by an accredited vascular ultrasonographer. Measurements to the femoral vessels (arterial and superficial venous) were taken bilaterally; at baseline and 5 minutes before the completion of each programme, while the device was still active. Parameters measured include, peak maximum velocity, vessel diameter, and volume flow. Estimations of these parameters were obtained for the entire waveform. Measurements were performed in duplicates to ensure reproducibility. Venous volume flow measurements using IPC devices are usually presented incorrectly accounting for the inflation cycle only, thereby significantly overestimating their true effect. Thus, taking into account the difference in the inflation/deflation cycle, calculations were performed for venous volume flow measurements according to the underlying formula, in order to obtain the exact volume flow per minute.

Venous Volume flow per minute

$$= \frac{\text{Inflate venous blood volume}}{60 \text{ seconds/inflation time}} + \frac{\text{Deflate venous blood volume}}{60 \text{ seconds/deflation time}}$$

7.4.3.4. Tolerance & Acceptance

At the end of each program, subjects were asked to evaluate their acceptance and tolerability to each device using a discomfort questionnaire, refer to Appendix 28. Discomfort was compared to a blood pressure cuff inflated around the upper arm. Subjects rated their discomfort levels using a visual analogue scale (VAS), by marking the level of the perceived pain using a 100 mm line, marked at one end “no sensation”

and at the other end “severe discomfort”. Verbal rating score (VRS) was also used to categorise the rating of the discomfort pain as:

- 1 = no sensation (other than muscles tensing and relaxing)
- 2 = minimal sensations
- 3 = mild discomfort
- 4 = moderate discomfort
- 5 = severe discomfort

At the end of the assessments, the subject’s deep veins were re-examined with duplex ultrasound to exclude the development of DVT.

7.5. Statistical Analysis

All assessments were acquired at baseline and at the end of each programme (30 minutes). As in the previous chapter, the baseline values obtained for each assessment performed was used as a reference range. Statistical analysis was performed using Minitab 16 software (Minitab Ltd, UK). Data shown in the results section represent the mean of data obtained from the 10 volunteers studied and are presented as median (IQR).

7.6. Results

7.6.1. Ultrasound Assessments

7.6.1.1. Mean Blood Volume Flow

Analysis of the data using linear models indicated that there is a highly significant difference in both venous and arterial blood volume flow between the devices ($p \leq 0.001$) refers to **Figure 56** & **Figure 57**. The values obtained for both venous and arterial blood volume flow of the devices tested are displayed as median (IQR) in **Table 26**.

Device	Venous Volume Flow mL/min Median (IQR)	Arterial Volume Flow mL/min Median (IQR)
Baseline – Without any device	123.5 (73.4)	197.5 (135.8)
geko™ T-1 High Pulse Width	163 (105.3)	244.5 (125)
geko™ T1- Low Pulse Width	129 (42.7)	170 (107.5)
IPCHF	118(72.7)	181.5 (70.5)
IPCKendall	115 (60.2)	158 (73)

Table 26: Comparison of Venous and Arterial Blood Volume Flow for the geko™ T-1 and IPC Devices.

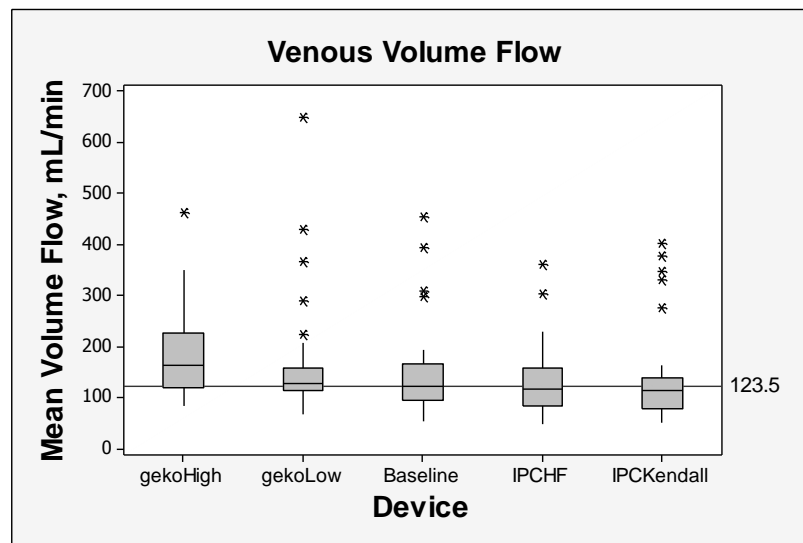


Figure 56: Mean venous blood volume flow measurement, $p \leq 0.001$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

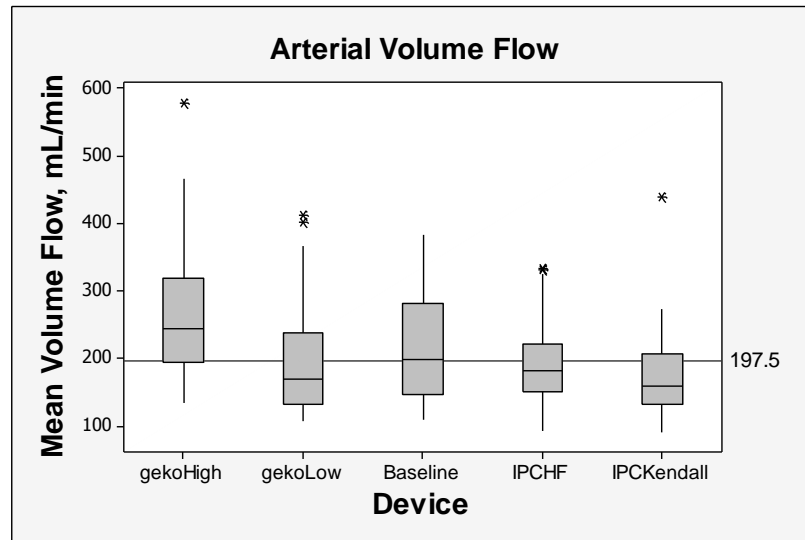


Figure 57: Mean Arterial blood volume flow measurement, $p \leq 0.001$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

Considering the average percentage change in comparison to the baseline for venous blood flow measurements, the highest average percentage change was achieved following the use of the geko™ T-1 device at higher pulse widths (33%), followed by geko™ T-1 device at a lower pulse widths (14%). Surprisingly, both IPC devices demonstrated similar values below baseline levels (-4%).

Equally, when examining arterial blood flow the highest average percentage change was obtained following the use of the geko™ T-1 device at higher pulse widths (30%). However, unlike the venous volume flow measurements, the use of the geko™ T-1 device at the lower pulse width reported a decrease in the percentage change below baseline (-7%) that was roughly similar to that reported following the use of IPCHF device (-9%); with the lowest percentage change reported for the IPCKendall device (-16%).

7.6.1.2. Mean Blood Velocity

Blood velocity measurements for IPC devices were made during both the inflation and deflation phase, taking into consideration the marked difference in the compression period between the geko™ T-1 device and the IPC devices. The geko™ T-1 device

accelerates consistently every second (1Hz) unlike the IPC devices, which accelerate only during the inflation period, refer to **Figure 58, Figure 59 & Figure 60**.

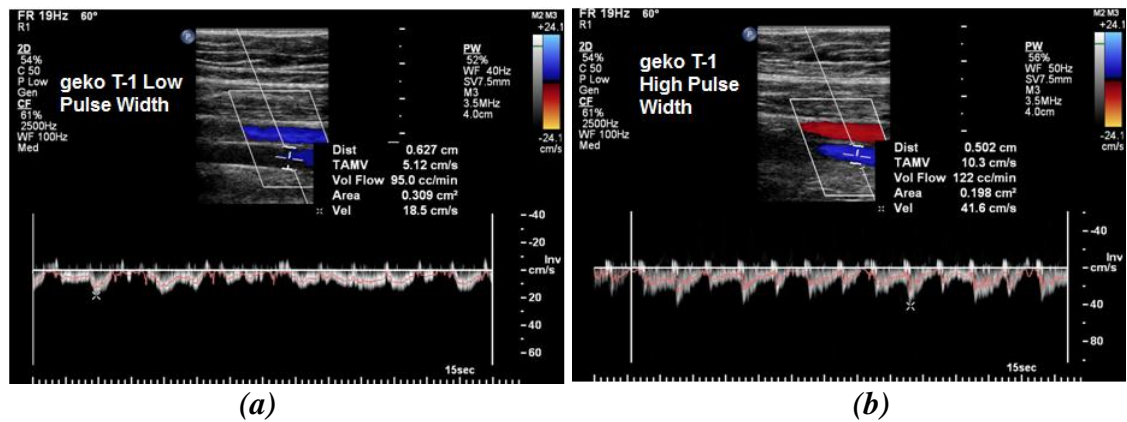


Figure 58: Colour Flow Duplex Ultrasound of the geko™ T-1 Device at High and Low Pulse Width Settings. A consistent acceleration is demonstrated with the geko™ T-1 device at both pulse width settings. A greater augmentation in blood velocity is seen when using the geko™ T-1 device at a higher pulse width settings (b) in comparison to the lower pulse width setting (a).

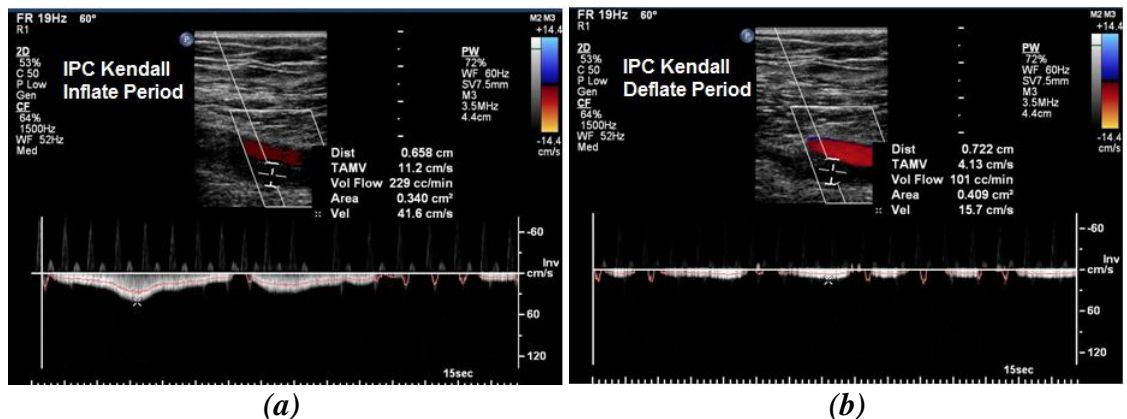


Figure 59: Colour Flow Duplex Ultrasound of IPC Kendall™ Device during Inflation and Deflation Period. The Kendall™ device displays a greater acceleration during the inflation period as shown in (a), marked by a greater mean velocity. This is compared to a lower velocity during the deflation period as shown in (b).

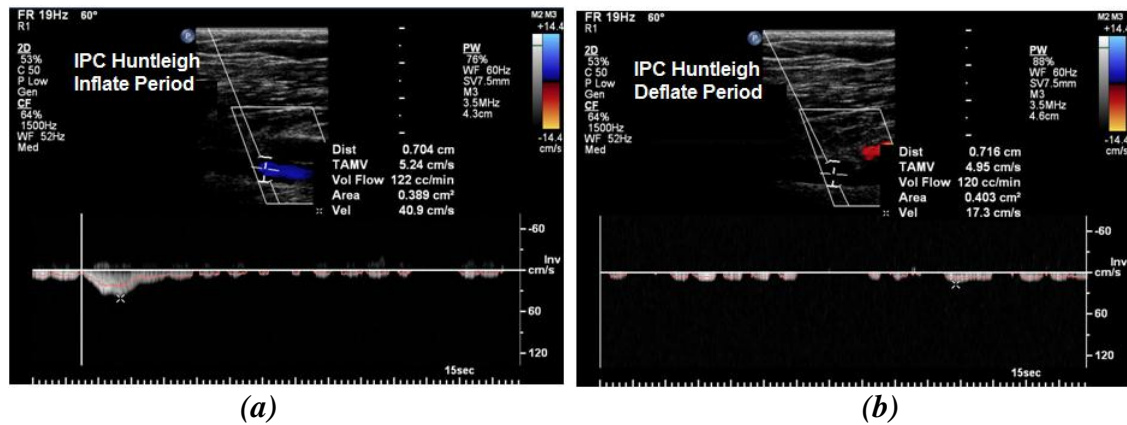


Figure 60: Colour Flow Duplex Ultrasound of IPC Huntleigh™ Device during Inflation and Deflation Period. The Huntleigh™ device accelerates only during the inflation period as shown in (a), marked by a greater mean velocity. In comparison, a lower velocity is seen during the deflation period as shown in (b).

Results obtained following analysis of variance showed a highly significant difference in blood velocity between the devices, $p \leq 0.001$. Analysis of venous velocity measurements, revealed a substantial increase by 174% following the use of the geko™ T-1 device at higher pulse widths, which was equivalent to the IPC devices; 166% and 143% for IPCHF and IPCKendall respectively, refer to **Figure 61**. A 73% increase was reported following the use of geko™ T-1 at the lower pulse width. Although this is lower than the performance of both the geko™ T-1 device at higher pulse widths and IPC devices during the inflation period, it is still considered to be much better than the performance of the IPC devices during the deflation period. During the deflation period, there was only an 11% increase following the use of IPCHF and a decrease by -9% following the use of IPCKendall.

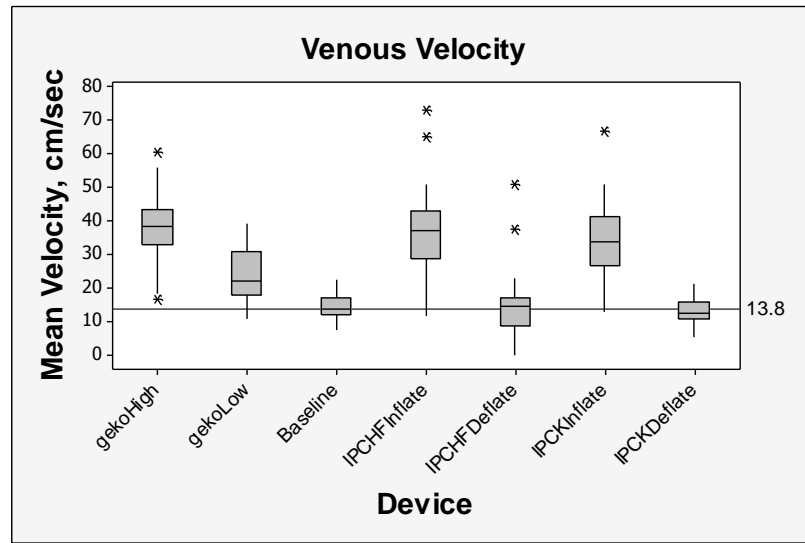


Figure 61: Mean Venous Velocity, $p \leq 0.001$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

The median values reported following the use of the geko™ T-1 device at high settings were equivalent to that of the IPC devices during the inflation phase. The median (IQR) for venous and arterial velocity measurements are displayed in **Table 27**.

Device	Venous Velocity cm/sec Median (IQR)	Arterial Velocity cm/sec Median (IQR)
Baseline – Without any device	13.8 (5.4)	83.15 (24.23)
geko™ T-1 High Pulse Width	38.3 (10.35)	98.25 (27.70)
geko™ T1- Low Pulse Width	22 (12.75)	84.75 (22.10)
IPCHF – Inflation Period	37 (14.25)	81.9 (20.40)
IPCHF – Deflation Period	14.7 (8.35)	79.7 (17.15)
IPCKendall- Inflation Period	33.7 (14.63)	80.3 (17.85)
IPCKendall-Deflation Period	12.6 (5.2)	85 (15.2)

Table 27: Comparison of Venous and Arterial Blood Velocity for the geko™ T-1 and IPC Devices.

Similarly, when measuring the arterial velocity, the highest increase reported was following the use of the geko™ T-1 device at higher pulse widths, where the velocity increased by 24% compared to the baseline. A small increase was reported for the geko™ T-1 device (2%) when used at lower pulse width. In comparison, a decrease was observed following the use of the IPCKendall by -1% during the inflation phase, and a

further decrease was reported following the use of IPCHF device by -4% during the inflation phase, refer to **Figure 62**.

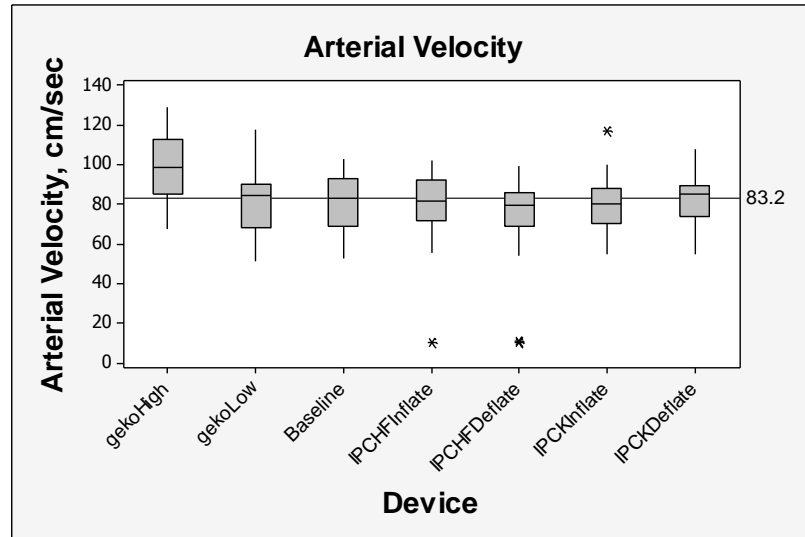


Figure 62: Mean Arterial Velocity, $p \leq 0.001$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

7.6.1.3. Mean Vessel Diameter

No significant difference in mean femoral vessel diameter was reported between the devices studied $p > 0.05$. Both venous and arterial measurements remained stable throughout the study refer to **Figure 63** & **Figure 64**. The median (IQR) range obtained for both the arterial and venous vessel diameters is displayed in **Table 28**.

Device	Venous Vessel Diameter, mm Median (IQR)	Arterial Vessel Diameter, mm Median (IQR)
Baseline – Without any device	7.78 (2.04)	6.58 (1.07)
geko™ T-1 High Pulse Width	7.61 (2.40)	6.43 (1.00)
geko™ T1- Low Pulse Width	7.46 (2.46)	6.56 (1.30)
IPCHF – Inflation Period	7.65 (1.8)	6.33 (0.86)
IPCHF – Deflation Period	7.6 (1.52)	6.33 (0.80)
IPCkendall- Inflation Period	7.18 (1.58)	6.17 (0.98)
IPCkendall-Deflation Period	7.5 (1.81)	6.33 (0.92)

Table 28: Comparison of Venous and Arterial Vessel Diameter for the geko™ T-1 and IPC Devices.

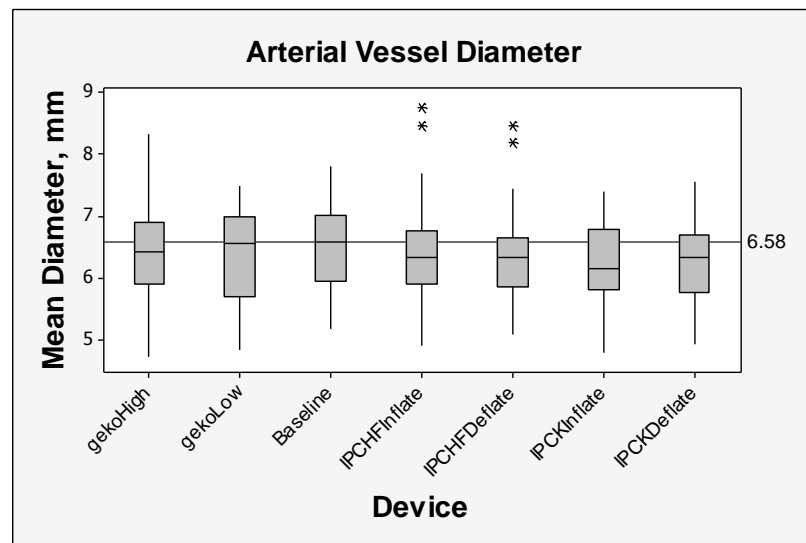


Figure 63: Mean Arterial Vessel Diameter, $p > 0.05$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

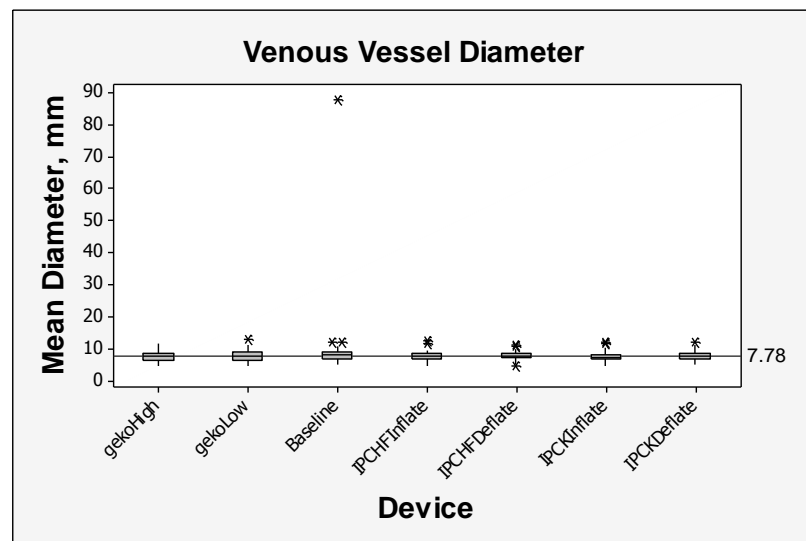


Figure 64: Mean Venous Vessel Diameter, $p > 0.05$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

7.6.2. Skin Microcirculatory Assessments

Microcirculatory assessments using Laser Doppler flowmetry (LDF) showed a highly significant difference between the devices, $p \leq 0.001$. The geko™ T-1 device at both

higher and lower pulse widths showed a substantial increase in microcirculatory blood velocity by 394% and 345% respectively. This is compared to a 59% increase achieved following the use of IPCKendall and 44% increase following the use of IPCHF device, refer to **Figure 65**. The median (IQR) range obtained is displayed in

Device	Laser Doppler Flowmetry, flux units Median (IQR)
Baseline – Without any device	9.45 (7.61)
geko™ T-1 High Pulse Width	35.46 (24.26)
geko™ T1- Low Pulse Width	27.13 (24.92)
IPCHF	6.67 (7.89)
IPCKendall	6.71 (12.58)

Table 29: Comparison of Skin Microcirculatory Velocity obtained using Laser Doppler Flowmetry for the geko™ T-1 and IPC Devices.

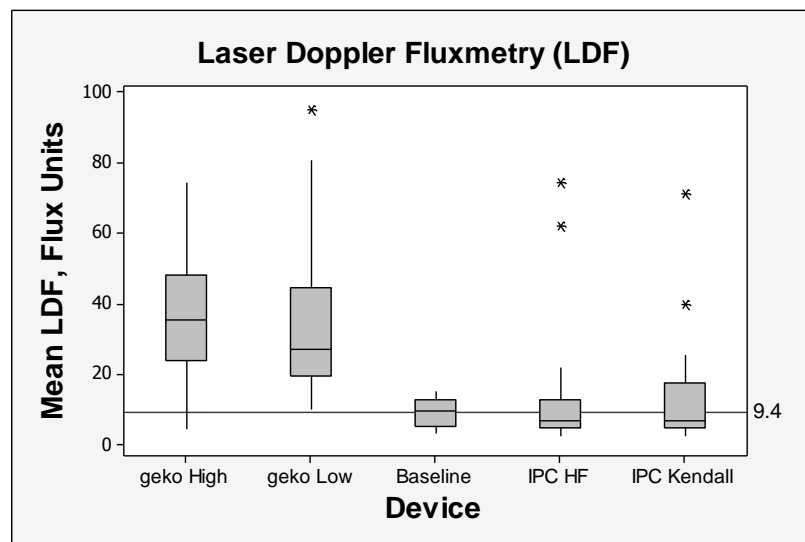


Figure 65: Mean Laser Doppler Flowmetry, $p \leq 0.001$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

7.6.3. Safety Assessments

No significant difference was reported between the devices following the measurement of blood pressure, transcutaneous tissue oxygen (TcPO₂), tissue oxygen saturation (SPO₂) as

well as heart rate, $p > 0.05$. All measurements remained equally stable throughout the study refer to **Figure 66**, **Figure 67**, **Figure 68** & **Figure 69**.

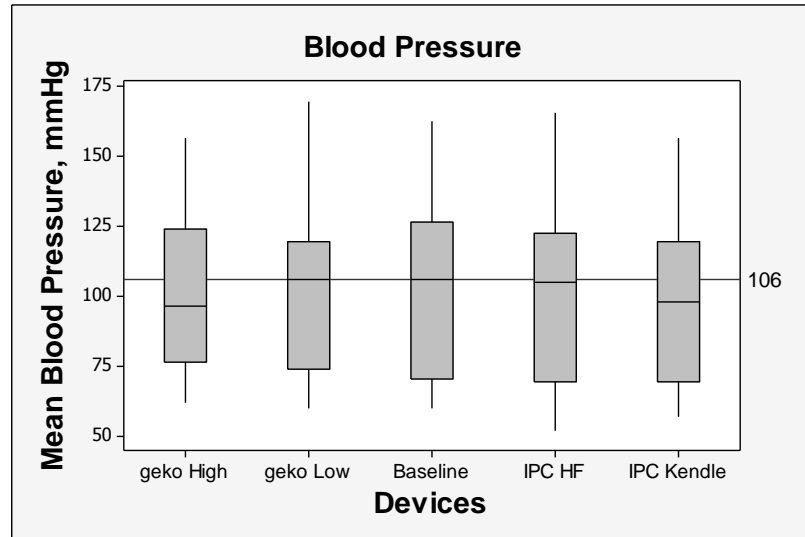


Figure 66: Mean Blood Pressure, $p > 0.05$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

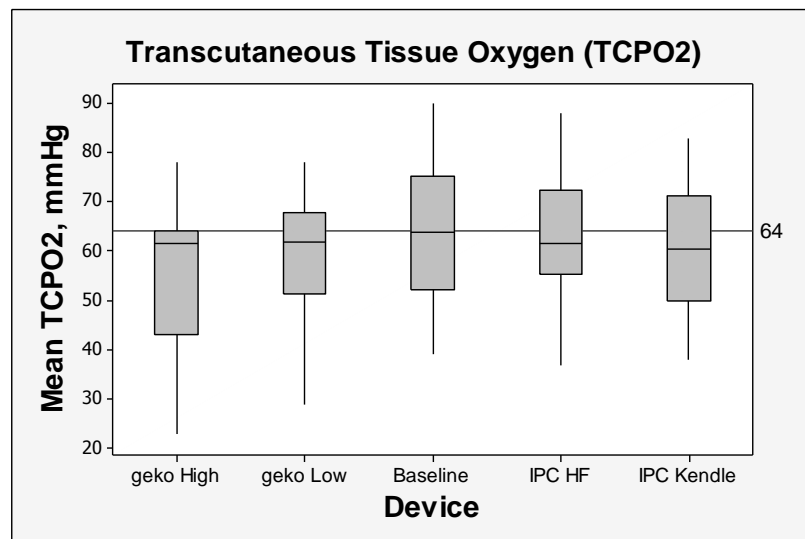


Figure 67: Mean Transcutaneous Tissue Oxygen, $p > 0.05$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

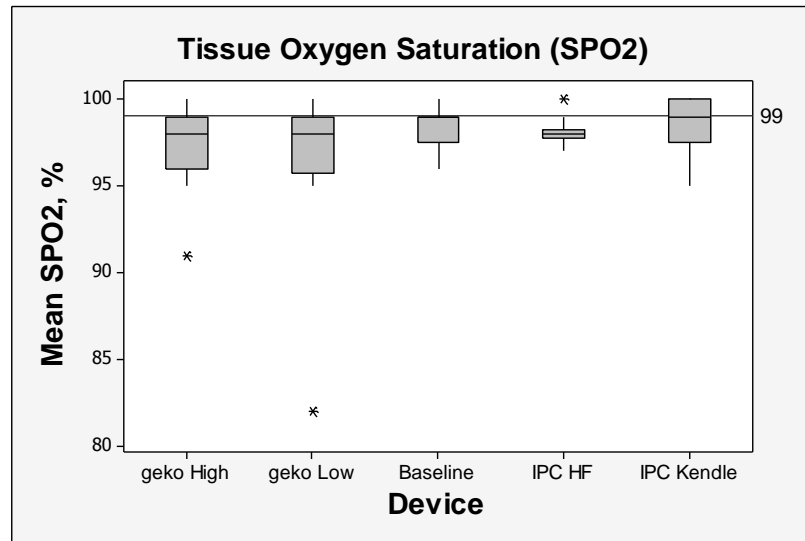


Figure 68: Mean Tissue Oxygen Saturation, $p > 0.05$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

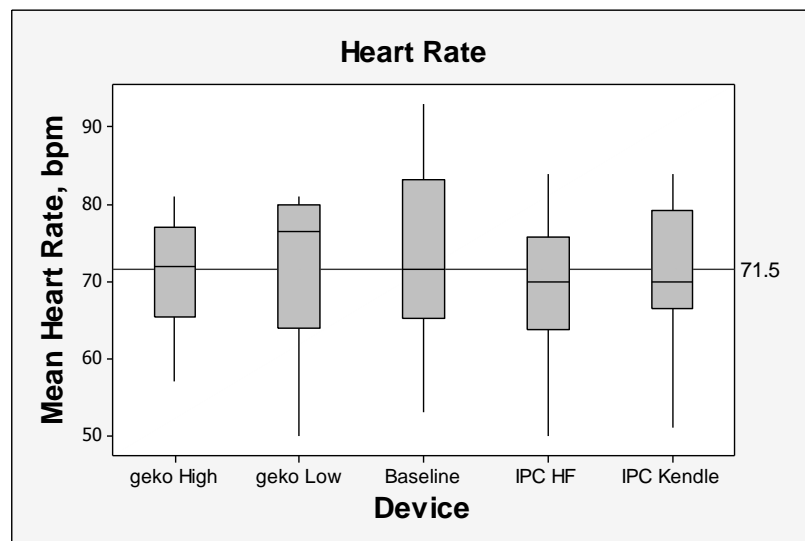


Figure 69: Mean Heart Rate, $p > 0.05$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

7.6.4. Discomfort Assessments

Analysis of the discomfort levels reported following the use of each device was not significant using the visual analogue scale, $p > 0.05$, but showed a statistically significant difference using the verbal rating score, $p \leq 0.05$ refer to **Figure 70** & **Figure 71**. Using the verbal rating score, the discomfort level following the use of the geko™ T-1 device at a higher pulse width was rated at mild discomfort as compared to the other devices studied, which were rated at a minimal sensation.

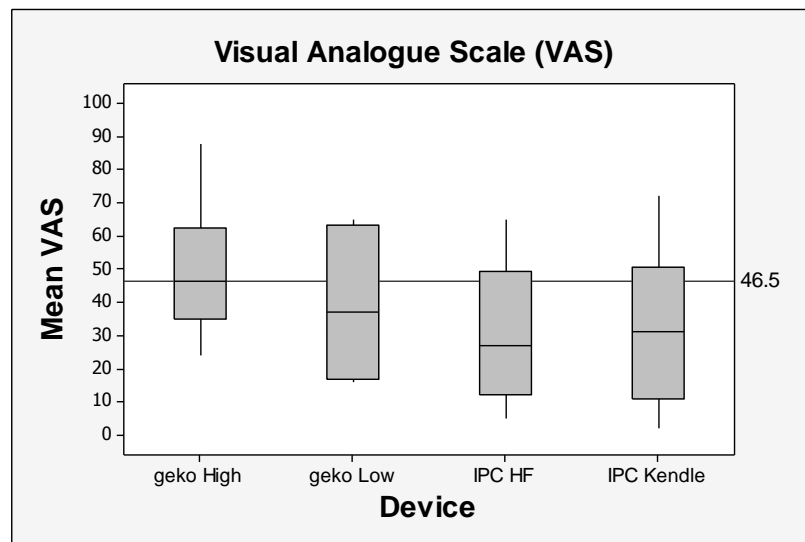


Figure 70: **Visual Analogue Scale, $p > 0.05$.** Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

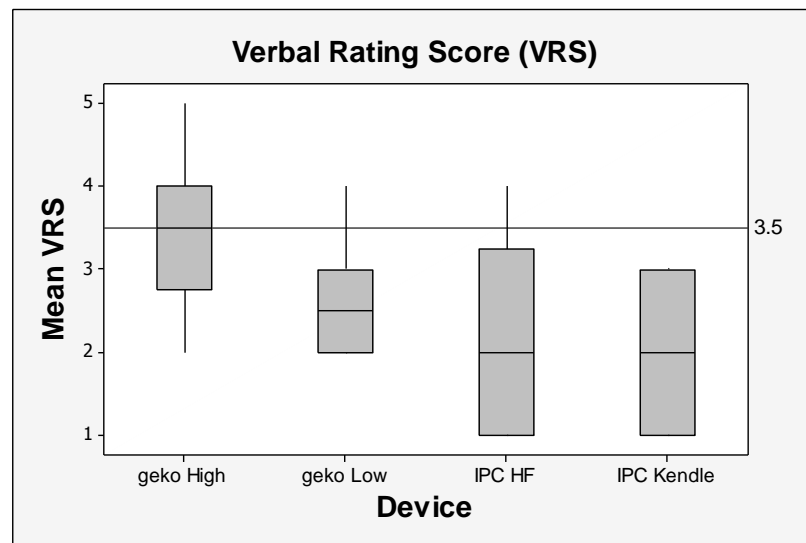


Figure 71: Verbal Rating Score, $p \leq 0.05$. Bottom of the box = 25th percentile, centre of the box = median (50th percentile), top of the box = 75th percentile, vertical lines = extremes of values within 1.5 times the IQR. Asterisks = outliers. Middle line across = Baseline median.

7.7. Discussion

The present study compares the effectiveness of a novel electrical nerve stimulation device with two leading IPC devices commonly used in hospital settings. Maintaining adequate peripheral blood flow in the lower limb is essential to prevent venous stasis, hence preventing the formation of DVT. The devices in this study may be considered effective in preventing venous stasis; however they differ in the magnitude of increase in lower limb blood perfusion (Morris and Woodcock, 2004, Tucker et al., 2010, Westrich et al., 1998).

Based on the analysis of the data obtained from 10 healthy subjects, it can be inferred that the novel device studied (geko™ T-1), is superior to IPC Huntleigh Flowtron™ and IPC Kendall SCD™ devices in enhancing blood flow in the lower limbs. This is evident from the substantial significant increase in the femoral venous and arterial blood volume flow by ~ 30% following the use of the geko™ T-1 device especially at higher pulse widths. Although the volume flow increase following the use of the geko™ T-1 at lower pulse widths is less than that of the higher pulse widths (14%), it is still much greater than that

reported following the use of the IPC devices, where a decrease in volume flow was observed. The blood flow measurements performed in the majority of electrical stimulation studies are mostly for venous measurements hence, arterial data is absent. Thus, when comparing the venous volume flow results obtained in this study with results of recent studies performed investigating different electrical stimulation techniques, similar results were obtained. For instance, a greater volume flow was reported by (Broderick et al., 2010b) following stimulation using a novel skin surface electrodes placed over motor points of the calf muscle at a frequency of 36Hz. Similarly, an increase in volume flow was also demonstrated when investigating another novel electrical stimulation technique by (Griffin et al., 2010), however the increase was only significant at high stimulation rates with short contraction time intervals. Therefore, it appears that the stimulation evoked by the novel technology at lower frequencies compared with the available electrical stimulation techniques, is more efficient in driving a greater blood flow through the femoral blood vessel.

Measuring arterial peak velocities, demonstrated that the use of the geko™ T-1 device especially at higher pulse widths is more effective than IPC devices in producing an increase in the femoral artery and vein. This is in comparison to a fall in arterial velocity following the use of IPC devices, which may be of concern especially in patients with peripheral arterial disease. Furthermore, our data shows that the geko™ T-1 device is as efficient as the IPC devices during the inflation period in increasing peak venous velocity. Those results are in agreement with the data obtained by (Izumi et al., 2010) and (Czyrny et al., 2010) where a higher increase in the femoral venous velocity was reported following electrical stimulation as compared to IPC. Although the use of the geko™ T-1 device at lower pulse widths demonstrated lower increase (73%) as compared to an increase by 174% at higher pulse widths, it is still considered more effective than the IPC devices during the deflation period. The use of the IPC devices showed an increase by 11% only for IPCHF and a fall by 9% for IPCKendall. The results obtained further confirm that the frequent acceleration in velocity for the geko™ T-1 device (every second) compared to an acceleration every minute by the IPC devices is more effective in enhancing blood flow, hence preventing venous stasis.

Peak venous velocities are considered a very popular measure by some of the manufacturers to indicate the efficacy of their systems. Although there is a scarce evidence that systems that produce higher velocities during compression, yield low DVT rates, a study published by Proctor et al suggested the opposite (Morris and Woodcock, 2004, Proctor et al., 2001). Published data have shown that the use of IPC devices at pressures of 40 mmHg, achieves a calf compression between 35 to 60cm/sec (Flam et al., 1996, Keith et al., 1992, Morris and Woodcock, 2004, Ricci et al., 1997, Whitelaw et al., 2001). The results obtained from our study are in agreement with those figures, as the mean velocity range obtained following the use of IPCHF and IPCKendall during the inflation phase is 36 and 33cm/sec respectively.

Laser Doppler flowmetry measurements, which reflect the microcirculatory blood velocity in the skin, increased substantially by ~ 370% following the use of the geko™ T-1 device in comparison to a modest 59% and 44% increase following the use of the IPCHF and the IPCKendall devices respectively. Interestingly, studies have revealed the efficacy of electrical stimulation in wound care by increasing blood flow and enhancing tissue oxygenation, thereby targeting inflammation and proliferation phases of wound healing (Feedar et al., 1991, Mulder, 1991, Weiss et al., 1990). Thus, the vast increase in laser Doppler flowmetry values, together with the tissue oxygen results, suggests the potential benefits of the geko™ T-1 device in lower limb wound care. However, further studies are needed to confirm this.

Furthermore, safety parameters were measured to compare the safety of the geko™ T-1 device to the IPC devices. Pulse oximetry measurements confirmed a stable heart rate and saturated oxygen levels throughout the study. No significant differences ($p > 0.05$) in heart rate and mean oxygen saturation were reported between the geko™ T-1 device and IPC devices. The stable heart rate was also reported in a recent study using a novel electrical stimulation at a frequency of 36Hz (Broderick et al., 2010b). This further provides a non-subjective assessment of subject tolerance and potential compliance. Transcutaneous tissue oxygen levels also remained stable throughout the study. Furthermore, blood pressure measurements showed no differences. This again is a significant indicator of the safety of the device. Duplex ultrasound measurements of the

arterial and venous femoral vessel diameter, also showed no significant change in the mean vessel diameter throughout the study, which confirms the validity of the ultrasound volume and velocity measurements.

Pain due to high intensity settings has been a major hurdle to the development of electrical stimulation technology, especially direct muscle stimulation. In many cases this has limited their application and clinical usage to the anaesthetised patient. Likewise, the immobilisation, discomfort, bulkiness, excessive heat and sweating associated with the use of IPC devices has always been a major drawback to their use despite their effectiveness in preventing DVT. Discomfort assessment by verbal rating score in this study demonstrated that the use of the geko™ T-1 at higher pulse widths is associated with only a mild discomfort. This is in comparison to a minimal sensation perceived following the use of IPC devices as well as the geko™ T-1 device at a lower pulse width. It is essential to note that the devices were active for a short period of time (30 minutes), thus the mild discomfort following the use of the geko™ T-1 device at higher pulse widths, could be minimal if the duration is increased as the subject might develop a greater tolerability.

A key limitation to this study is the small population number. The present study was conducted on a small number of healthy subjects, so the results obtained can hardly be extrapolated to patients. Therefore, further studies are required on larger population of patients to confirm the results obtained. Moreover, as all examinations in the present study were performed with the subjects lying supine, it might be useful to conduct additional studies at different postures to further assess the compliance to the device and the effectiveness in enhancing blood flow.

In conclusion, the present study is the first to compare the effectiveness of a portable self-contained electrical stimulation device to the widely used intermittent pneumatic compression systems. The findings of the study demonstrate the potential benefits of using electrical nerve stimulation in improving circulatory dynamics in the arterial, venous and microcirculatory vasculature of the lower limb. The stimulation applied was perceived by healthy subjects as acceptable, as only a mild/minimal discomfort was

associated with its use. We propose that the novel device has a significant potential for the development into an easy-to-use, pain-free DVT prophylaxis device, with a potential use in the management of several other vascular disorders.

Chapter 8. General Discussion

Deep vein thrombosis is a medical condition associated with the formation of a thrombus in the deep veins, usually in the lower leg. The main physiological contributing factors for the development of DVT as described by Virchow's Triad are hyper-coagulability, endothelial damage and venous stasis. The natural mechanism for the prevention of venous stasis in an ambulatory healthy individual is the process of walking. Decreased mobility increases the risk of DVT due to inadequate pumping action resulting in insufficient venous return to the heart.

Prophylaxis for DVT is divided into pharmacological and mechanical methods. Pharmacological methods are effective, but are contraindicated in certain cases where there is a high risk of bleeding. Mechanical methods are mainly graduated compression stockings, intermittent pneumatic compression devices and electrical stimulation. Published data on the effectiveness of neuromuscular electrical stimulation in preventing DVT dates back to 1972, where electrical impulses were shown to increase blood flow response in the lower extremities (Nicolaides et al., 1972). Studies on the usefulness of electrical stimulation continued in the subsequent years, increasing the scope of the technique not only in the prevention of DVT, but for a wide range of conditions (Currier et al., 1986, Czynny et al., 2010, Faghri et al., 1997, Kaplan et al., 2002, Kostin, 1993, Velmahos et al., 2005). However, the unbearable pain associated with its use has limited its use to surgical procedures under general anaesthesia. Painless treatment was therefore an important goal considered during our investigations.

The rationale for carrying out the studies described in this thesis was to investigate the effectiveness of a novel neuromuscular electrical stimulation device (geko™ T-1) that could be used potentially in the management of DVT as well other disorders associated with abnormal blood flow.

The studies described in this thesis were all performed on healthy subjects. The primary objective of the studies was to investigate the effectiveness of the geko™ T-1 device in enhancing lower limb blood perfusion using colour flow duplex ultrasound and laser

Doppler flowmetry. The secondary objective was to investigate whether the enhancement of venous return results in an improved cardiac performance. Additionally, to assess the effect of the geko™ T-1 device on specific blood coagulation parameters such as clotting time, tissue plasminogen activator antigen, von willebrand factor antigen, prostacyclin and endogenous thrombin potential. Other objectives were to evaluate the safety of the geko™ T-1 device investigated by assessing blood pressure, heart rate, oxygen saturation and transcutaneous tissue oxygen tension. Furthermore, subjects' acceptance and tolerance to, the novel device was assessed. Finally, an evaluation of the effectiveness of the device in relation to the most commonly used devices for mechanical prophylaxis (IPC devices) in hospital settings was preformed.

8.1. Electrical Stimulation Effect on Cardiac Performance

The effect of the novel device on cardiac performance was initially investigated on healthy subjects lying supine. Cardiac performance was evaluated using echocardiography by measuring cardiac output, ejection fraction, as well as diastolic function. Analysis of the data acquired from the first study demonstrated that, in comparison to the baseline measurements, the geko™ T-1 device significantly increased cardiac output. Although for statistical efficiency, the data were analysed untransformed, percentage changes can make the results more accessible (Vickers, 2001). In percentage terms, the geko™ T-1 increased cardiac output by 4 to 6%. These results compliment the results obtained by (Faghri et al., 1997), who demonstrated a 24% increase in cardiac output following electrical stimulation compared to a 14% fall following the use of a sequential compression device. The reported increase in cardiac output could be due to the direct activation of the calf muscle pump, which causes a complete emptying of both venous beds and sinuses (Faghri et al., 1997). Cardiac output, which is the amount of blood ejected by the left ventricle in the time interval of one minute is directly proportional to heart rate (Nursecom, 2004). Thus, as heart rate increases, so does cardiac output. The increase in cardiac output obtained could be due to exposure to a foreign sensation, which could have resulted in the increase of heart rate. However, as heart rate was not monitored throughout the study period, it was difficult to confirm this. Findings from studies using electrical stimulation have demonstrated an increase in heart rate

(Broderick et al., 2010b). Contractions of the calf muscle evoked by electrical stimulation, stimulates the sympathetic nervous system that leads to increased heart rate and further increases the contractility of the heart muscle (Broderick et al., 2010b). Furthermore, the data acquired from this study also demonstrate that the geko™ T-1 device investigated has a slight influence on ejection fraction, and does not alter the filling pattern in the left ventricle of the heart in healthy volunteers.

8.2. Electrical Stimulation Effect on Tissue Perfusion

Lower limb blood perfusion was of prime concern throughout all the studies. Initially, when performing the cardiac study, assessment of blood perfusion was made by measuring blood flow parameters in the femoral artery. Based on the results acquired from the study, in percentage terms a significant increase of greater than 50% was obtained when measuring arterial blood volume flow in comparison to the baseline.

Further analysis of lower limb blood flow was performed throughout the subsequent studies by measuring blood perfusion in both the femoral artery and superficial femoral vein. Data obtained from the THRIVE study showed a significant augmentation in arterial blood volume flow following stimulation. Based on the data obtained from the stimulation study, the highest increase in arterial volume flow was noted at the first hour of the study, where a substantial increase from 177mL/min at baseline to 289mL/min was demonstrated. Arterial volume flow then decreased slightly throughout the study period, although values remained higher than the baseline. In contrast, a significant drop in arterial volume flow was demonstrated throughout the control study period, with the greatest drop (21%) seen at the first hour of the study. The change observed at the first hour of the study could be stress related. Therefore, it is clear from the results obtained that the application of the novel electrical stimulation device augments arterial volume flow, which suggests a reduction in peripheral vascular resistance. The augmentation in arterial blood volume flow was further confirmed when comparing the novel electrical stimulation device with IPC devices. An increase of 30% in arterial blood flow following the use of the geko™ T-1 device in comparison to the baseline was demonstrated, this was compared to a drop when using the IPC devices.

Similarly, the significant increase in blood volume flow was not only apparent in the artery, but also in the superficial femoral vein. Following stimulation venous blood volume flow increased significantly throughout the study period in comparison to the baseline. As compared to similar existing muscle electrical stimulation method (MEST) that show only 25% increase in the femoral venous blood flow (Zhao et al., 2004), the venous blood volume flow results obtained in our studies were exceptionally high. The highest increase was reported during the THRIVE study following 3 hours where the blood volume flow increase was close to six fold in comparison to the baseline. The significant augmentation reported was further confirmed when comparing the geko™ T-1 device to the IPC devices, as the geko™ T-1 device was found to be superior in increasing venous blood volume flow by 33% in comparison to the baseline. This suggests that the geko™ T-1 device is highly effective in enhancing blood flow through the femoral vein. Similar results were reported in a study comparing a novel electrical stimulation technique with IPC devices. In this study, a greater increase in blood volume flow following electrical stimulation in comparison to IPC devices was demonstrated (Izumi et al., 2010).

In addition to the significant augmentation in blood volume flow, the studies carried out demonstrated that the geko™ T-1 device increases peak venous velocities at the femoral vein that by far exceeds the baseline measurements. Data from the THRIVE study; demonstrated a substantial increase in venous velocity (221%) following stimulation. Furthermore, the geko™ T-1 device causes an equivalent increase (38 cm/sec) in venous velocity to that of the IPC devices during the inflation period. However, the geko™ T-1 device was superior to the IPC devices during the deflation period in maintaining venous velocity. The findings of our study, although differing in the magnitude of the increase in velocities, are again similar to those reported by (Izumi et al., 2010), who also reported a greater increase in peak venous velocities (97 cm/sec) following electrical stimulation as compared to a lower increase using IPC (65cm/sec).

Studies have shown that an increase in venous volume and velocity causes venous emptying and facilitates clearance within the soleal sinuses and valve cusps, which are the most common sites for DVT formation (Flanc et al., 1968, Nicolaides et al., 1971).

The soleus sinuses are the largest veins draining the muscle and are responsible for the largest volume of flow from the muscle into the deep veins upon contraction (Broderick et al., 2010b). Published studies have reported that the IPC devices are not capable of entirely eliminating venous stasis, as the blood is being pushed past the venous sinuses via the deep veins, leaving behind pooled blood in the sinuses (Faghri et al., 1997, Laverick et al., 1990). The use of electrical stimulation methods may therefore be more effective than IPC devices, as the contractions of the calf muscle pump results in the complete emptying of the soleal sinuses (Laverick et al., 1990).

In comparison to the venous velocity, arterial peak velocity was only significantly raised in the cardiac study and when comparing the geko™ T-1 device with IPC devices. Interestingly, in both studies, following the use of the geko™ T-1 device arterial velocity increased by 24%. This confirms the reproducibility of the measurements and suggests that the device might be useful in patients with peripheral arterial disease. However, further studies are needed to confirm this.

Laser Doppler flowmetry, which is a reflection of the microcirculatory blood velocity in the skin, was another mean of confirming the effectiveness of the geko™ T1 device. The data acquired from all the studies presented, showed a substantial increase in microcirculatory velocity that by far exceeds the baseline measurements. For instance, in the first study, following stimulation, laser Doppler flowmetry increased from 7.71 flux units at baseline to 117.9 flux units. The increase was confirmed during the stimulation study, which unlike the control study showed a highly significant augmentation in microcirculatory velocity in the stimulated leg from 4.66 flux units at baseline to 75.8 flux units. This was again confirmed when comparing the geko™ T-1 device to IPC devices, as a large increase of ~370% was reported when using the geko™ T-1 device in comparison to a modest increase of ~40% following the use of the IPC devices. The findings confirm the validity of the laser Doppler measurements obtained, and also adds to the potential benefits of the geko™ T-1 device in the reduction of peripheral vascular resistance. This might be of benefit in wound healing. It has been shown that electrical stimulation affects the inflammation and proliferation phases by increasing blood flow and enhancing tissue oxygenation. Hence, electrical stimulation has been widely used as

an additional therapy in wound care management for pressure, venous and ischemic ulcers (Feedar et al., 1991, Kloth and Feedar, 1988, Mulder, 1991).

Based on the ultrasound and laser Doppler flowmetry findings in the studies presented in this thesis, a summary of the effect of the geko™ T-1 device on blood flow as it passes through the systemic circulation is displayed in **Figure 72**. Results demonstrated that the geko™ T-1 device causes an augmentation in blood velocity when measured at the femoral artery and superficial femoral vein. An augmentation was also demonstrated at the capillary level when measuring the microcirculatory velocity as confirmed by the laser Doppler flowmetry results. This suggests that the electrical stimulations increase capillary recruitment by enhancing the activity of resting capillaries.

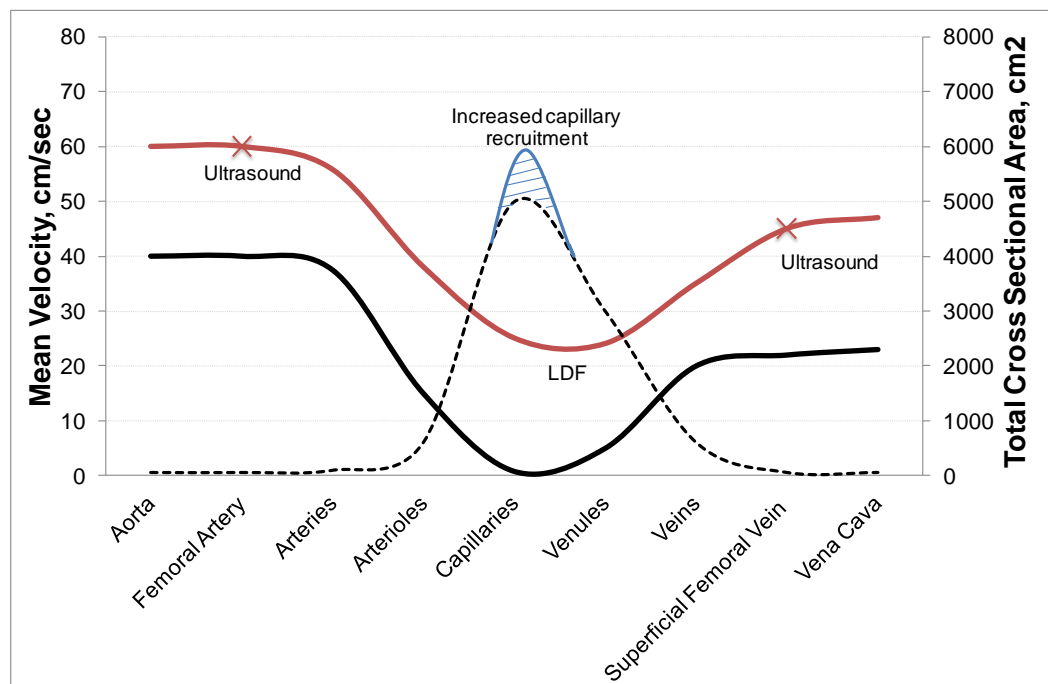


Figure 72: The effect of geko™ T-1 Device on Blood Flow. The effect of the geko™ T-1 device (red line) on normal mean blood velocity (solid black line) and total cross sectional area (dashed line). The geko T-1 device also causes an augmentation at the capillary level as shown by the LDF measurements and the blue dashed curve, suggestive of an increased capillary recruitment.

8.3. Electrical Stimulation Effect on Blood Coagulation

Blood coagulation factors investigated throughout the THRIVE study were, tissue plasminogen activator antigen (tPA), von willebrand factor (vWF) as well prostacyclin levels. The systemic effects of electrical stimulation was reported earlier by (Nicolaidis et al., 1972), where muscle contractions was shown to increase the fibrinolytic activity in the blood. In the THRIVE study presented earlier, a significant difference was only reported with tissue plasminogen activator antigen. In comparison to baseline levels, a greater and a highly significant drop in tPA antigen levels was demonstrated throughout the stimulation study in comparison to the control. Tissue plasminogen activator antigen is usually present as a bound complex of active tPA and plasminogen activator inhibitor 1, PAI-1 (Comerota et al., 1997a, Nordenhem and Wiman, 1998). A decreased fibrinolytic activity is associated with increased levels of PAI-1 hence, increased levels of tPA antigen. Therefore, the drop in tPA antigen concentration in the plasma might suggest an increased fibrinolytic activity to break down any clots formed, which may also be correlated with a drop in PAI-1. To confirm the above reasoning, further assessments must be performed by measuring concentrations of PAI-1. Furthermore, studies have shown that, a decreased fibrinolytic activity is a predictor of myocardial infarction (Held et al., 1997, Juhan-Vague et al., 1996, Nordenham et al., 2005, Scarabin et al., 1998, Thogersen et al., 1998). Thus, the drop in tPA antigen caused by the novel electrical stimulation might also be of beneficial use for cardiovascular patients. However, further investigations are needed to confirm this.

Another blood coagulation factor assessed was von Willebrand factor (vWF), a protein secreted by endothelial cells. In comparison to the baseline, no significant difference in von Willebrand factor antigen levels was seen throughout both the stimulation and control study. Von Willebrand factor antigen is an indication of endothelial cell activation and dysfunction following venous occlusion (Scharrer and Vigh, 1993). Raised levels of vWF in the plasma is an indication of DVT as reported by (Nilsson et al., 1986). It is important to note here that the volunteers investigated were healthy; hence, the non significant change in vWF is justified. The findings obtained are supported by similar results from (Comerota et al., 1997b), who reported a drop in tPA antigen and no

significant change in vWF antigen levels following the use of IPC devices. This suggests that the geko™ T1-1 device induces a non specific disturbance of the endothelium. Similarly, when assessing prostacyclin throughout the stimulation study by measuring its stable marker, 6 keto PGF1 α , a non significant change was reported. This suggests that the electrical stimulation has no influence on prostacyclin synthesis.

The effect of the geko T-1 device on blood coagulation was investigated further through measuring clotting time using automated coagulation timer technology (ACT) and rotational thromboelastometry (Rotem®). A significant drop in clotting time was observed using both techniques. This suggests that there might be a systemic increase in coagulation taking place during the study period. However, the values reported were within the normal healthy range. Additional blood coagulation assessments were performed by measuring the endogenous thrombin potential (ETP), which is one of the main determinants of haemostasis and thrombosis (Vlieg et al., 2007). Studies have shown that an elevated level of ETP is usually associated with an increased risk for DVT (Eichinger et al., 2008, Vlieg et al., 2007). Assessment of other secondary components of the thrombin generation was also performed by measuring the peak height, lag time, and time to peak. No significant change in ETP levels together with peak height throughout the study period. Interestingly, a significant increase in lag time and time to peak was demonstrated throughout the study. This suggests that coagulation is taking place, however since other measurement techniques showed opposite results, it is difficult to confirm this.

8.4. Safety Evaluation of the Electrical Stimulation Device

Several parameters were measured to evaluate the safety of the geko™ T-1 device. Colour flow duplex ultrasound measurements of the superficial femoral vessel, showed no significant change in mean vessel diameter in comparison to baseline throughout all the studies. This supports the safety of the geko™ T-1 device investigated and confirms the validity of the ultrasound measurements performed. Furthermore, pulse oximetry measurements ensured constant monitoring of heart rate and saturated oxygen levels. Mean oxygen saturation remained constant and always above 97% throughout the study

period. Similarly mean heart rate remained stable and within the normal healthy range between 60 and 90 beats per minute. Based on the data analysis, no significant difference in mean oxygen saturation and heart rate was demonstrated. Those findings are in agreement with the findings reported by (Broderick et al., 2010b), who demonstrated a stable heart rate following the use of a novel electrical stimulation at a frequency of 36Hz. Similarly, when measuring transcutaneous tissue oxygen in the geko™ T-1 versus IPC comparison study, no significant difference between the devices was reported. The stabilised pulse oximetry and tissue oxygen results obtained are strong indicators for the potential use of the device in wound care.

Blood pressure findings were of great interest in the studies performed. In comparison to the IPC devices, significant change in blood pressure was observed when comparing the geko™ T-1 device with the IPC devices. Both systolic and diastolic blood pressure remained stable throughout the study period. Interestingly, a small but a statistically significant drop in systolic and diastolic pressure was noted following stimulation throughout the stimulation study period. However, measurements were still within the normal range. This is in comparison to a stable and an insignificant change throughout the control study. Such findings could imply that the geko™ T-1 device may be of importance to hypertensive patients. Further investigations possibly on hypertensive patients are needed to confirm this.

8.5. Tolerance and Acceptance of the Electrical Stimulation Device

Selecting a mechanical prophylaxis method requires equal consideration of both patient compliance and hemodynamic performance. It was argued that patient compliance is an important factor to consider in the selection of mechanical prophylaxis (Morris and Woodcock, 2004). At the end of the day, for the device to be effective it must be used regularly by the patient. Non-compliance is most commonly the result of discomfort or inconvenience. Significant pain due to high intensity settings has been a major obstacle to the development of electrical stimulation technology. Hence, the application and clinical use of the electrical stimulation technique have been limited to anaesthetised patients. It

was therefore necessary to assess the tolerance and acceptance to the novel neuromuscular electrical stimulation device throughout the studies. Analysis of verbal rating score results, showed that the stimulation applied was associated with only a mild discomfort, and a minimal sensation was perceived when using visual analogue scale. Those findings were again similar to the findings of (Broderick et al., 2010b), who also demonstrated a mild discomfort sensation following the use of a novel electrical stimulation method. However, it is important to note here that the electrical stimulation device was only active for a short period throughout the studies conducted. Therefore, it is possible that subjects might develop a greater tolerance and acceptance to the device when used for longer periods.

The non-compliance associated with the use of IPC devices has raised great concern. Intermittent pneumatic compression devices are usually cumbersome, expensive and require a fixed power source that confines the patient. In an observational study on the use of compression devices by traumatised patients, only 19% were fully compliant, and non compliance was associated with a higher rate of DVT (Cornwell et al., 2002). Compliance is not only associated with patients, but with nurses and other care providers who often disconnect the devices during the period of evaluation and fail to reconnect them again. (Bockheim et al., 2009). The geko™ T-1 device investigated is less susceptible to compliance issues, as the device is smaller, more discrete in comparison to IPC devices; it is also portable allowing the patient to ambulate freely without the need to disconnect the device. Recent assessments of the geko™ T-1 device on a number of post surgical patients in a specialised tertiary hospital in Australia, confirmed that the device is well tolerated by the patients and well received by the hospital staff (Medical, 2012).

Conclusion

Findings of the studies presented have demonstrated that the geko™ T-1 device is potentially beneficial. Firstly, due to its ability to increase blood flow at the venous, arterial as well as microcirculatory level. Secondly, due to exhibiting an enhanced fibrinolytic effect by decreasing levels of tissue plasminogen activator antigen.

Furthermore, the small size, portability and most importantly simplicity of use makes the geko™ T-1 device an essential prophylactic tool with a wide range of application possibilities. Although the study successfully reached its aims, the common limitation with all the studies presented in this thesis is the small numbers and the use of only young healthy volunteers. This might have influenced the degree of statistical significance of certain factors assessed in the study. Thus, there is a need to generalise and confirm the results obtained on a larger group of patients.

Chapter 9. Future Options

Investigations on the use of electrical nerve stimulation of the lower leg using the novel device (geko™ T-1) were encouraging. The geko™ T-1 device is believed to have significant implications for the prevention of DVT in hospitals, outpatients and community care settings. Furthermore, based on the findings obtained, there is a considerable potential for the device to be further applied to non DVT related applications to expand the range of indications.

After performing trials on healthy volunteers and confirming the safety and efficacy of the novel neuromuscular electrical stimulation device in enhancing lower limb blood perfusion, trials on selected patient population can be initiated. The objectives for carrying out future studies is to further confirm the results obtained on patients who are believed to be at a greater risk for developing DVT, or who suffer from a vascular or micro-vascular disorders. Additionally, to explore the effectiveness of the device in the management of conditions characterised by an abnormality in blood flow in comparison to commonly used mechanical methods. A summary of the studies, which have been planned and are in the progress of being implemented, are outlined below.

1. Study investigating the effectiveness of a novel electrical stimulation method on critical limb ischemic patients

Analysis of the findings obtained in the studies performed has clearly illustrated that the device can significantly stimulate lower limb blood flow in the arterial, venous and microcirculatory systems. Furthermore, the device seems to be well tolerated and no adverse events were observed. Such observations, provide the framework to study the effects of the geko™ T-1 device on critical limb ischemic patients. The primary objective of this study is to evaluate the effectiveness of the novel device in enhancing lower limb perfusion in limb ischemic patients. The secondary objective is to evaluate using non invasive techniques such as duplex ultrasound and laser Doppler flowmetry, the blood flow velocity and volume changes associated with the use of the geko™ T-1 at higher pulse width settings. Additional objectives are to explore the patients' acceptability and

tolerance through the use of a questionnaire and a scoring index. The geko™ T-1 device will be fitted bilaterally to their lower limbs in the supine position. During the stimulation period, non invasive blood flow and volume parameters will be measured. These include: colour flow duplex ultrasound scanning, pulse oximetry, laser Doppler flowmetry, transcutaneous tissue oxygen and transcutaneous carbon dioxide tension. Patients will also be asked to evaluate their acceptance and tolerance of the electrical stimulation sequences by the use of a Visual Analogue Scale and a Verbal Rating Score. Vital signs, including radial pulse, systolic and diastolic blood pressure will be performed prior commencing the stimulations.

2. A comparative study of the cardiovascular effects of intermittent pneumatic compression therapy and the geko™ electrical stimulator device in hip and knee replacement patients

As described earlier, patients undergoing orthopaedic surgery especially hip and knee replacement have an increased risk of developing DVT. This study is a multi-centred physiological response study. It is designed to be conducted in Stanmore, Middlesex, UK, at six sites involving a total of 72 patients (12 per site). The primary objective is to compare the effectiveness of the geko™ device with intermittent pneumatic compression in enhancing lower limb perfusion in patient volunteers prior to undergoing hip or knee replacement. The secondary objective is to compare and evaluate patients' tolerability and compliance of both systems. Each device will be active for a period of 60 minutes, the sequence of application of IPC and geko™ will be randomly selected. Bilateral duplex ultrasound assessment to the femoral artery and superficial femoral vein will be performed at rest and with the device active at 30 and 60 minutes period. A final scan will also be performed 30 minutes following the removal of each device. Patients will then be asked to evaluate their acceptance and tolerability to the devices applied using a verbal rating score and a visual analogue scale.

3. A pilot study investigating the effectiveness of the geko™ device in post-operative total hip replacement patients

The primary objective of the study is to compare the effectiveness of the geko™ device in improving venous circulation with A-V-Impulse foot compression device following orthopaedic surgery. The secondary objective is to compare and evaluate patients tolerability and compliance of both systems investigated. Furthermore, to observe and asses improvement in mobility following application of the devices investigated. The study is set to take place at the Freeman Hospital, Newcastle, UK. It will include 20 patients who have undergone total knee replacement and 20 patients who have undergone hip replacement. In addition to the chemical prophylaxis treatment, each device will be applied bilaterally for a period not exceeding 4 hours. Comparative blood flow analysis using colour flow duplex ultrasound to the femoral blood vessels will be performed at baseline and hourly. Comparative acceptance and compliance will then be evaluated using verbal rating score and visual analogue scale.

4. A randomised control trial to evaluate the effect of the geko™ T-1 device on measures of patient recovery following total hip replacement

Patients recovery following surgical procedures such as total hip replacement involves minimising the negative impact of surgery, so that patients can be pain free and functionally able to return to their preoperative state as quickly as possible. This study is a parallel group randomised controlled trial. The primary objective of this study is to evaluate the effectiveness of using geko™ T-1 device in addition to standard treatment, in comparison to using standard treatment alone on measures of patient recovery following total hip replacement. The secondary objective is to evaluate patient tolerability and compliance when using the geko™ T-1 device. The study is set to take place at the Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust, Bournemouth, UK and will involve a total of 24 patients (12 per group). Patients will be randomised pre-operatively to receive either standard treatment alone or standard treatment with the addition of using the geko™ T-1 device. Patients on the intervention group will be instructed to use the geko™ T-1 device when resting supine or when sitting

for at least 4 hours per day following surgery and until 6 weeks. Markers of post operative stress response, recover, and function after surgery will be measured on every post-operative day whilst in hospital and also in the out-patient clinic following 2 and 6 weeks post-operatively. Patients will also be asked to evaluate their tolerability and acceptance of using the geko™ T-1 device using verbal rating score and visual analogue scale.

5. The efficacy of the geko T-1 device in promoting the blood flow in a plaster cast as a way of offering mechanical DVT prophylaxis

Patients in a plaster cast are at risk of developing DVT. Chemical prophylaxis is traditionally used for those at highest risk. However, the daily administration of injections of heparin or warfarin can be quite exhausting and expensive. Mechanical devices available are also impractical and cannot be fitted with a cast. The geko™ T-1 device is therefore believed to offer a new approach to thromboprophylaxis. The geko™ T-1 device causes calf muscle contraction, which expresses blood from the deep venous system. Improving venous flow is known to reduce the risk of DVT, which is a common problem in orthopaedic patients and can affect patients in a plaster cast. Thus, the geko™ T-1 device can be applied to patients with a below knee cast. The study is an open label single centred study. It is set to take place at the Southampton University Hospitals Trust, Southampton, UK and will involve 10 volunteers. The primary objective of this study is to examine the blood flow characteristics of the deep venous system in the leg using colour doppler ultrasound and to investigate how the flow is modified by the application of a plaster with a geko™ T-1 device in healthy volunteers. The secondary objective is to evaluate microcirculatory blood flow changes, using laser Doppler flowmetry and transcutaneous tissue oxygen monitors. Additional objectives are to explore the participants' acceptability and tolerance through the use of verbal rating score and a visual analogue scale and to assess the pressure exerted on the leg at the points of contact during muscle contraction in order to understand any potential risk of tissue damage caused by repeated pulsing. A randomly selected leg will be immobilised by each of the following methods in sequence: Aircast-type boots (devices analogous to ski boots used to immobilise fractures), Plaster cast extending from the forefoot to above the knee and

plaster cast extending from the forefoot to below the knee. Bilateral blood flow measurements will be made to the lower limbs with the volunteer lying down, with legs elevated and standing. Assessments will be made after 10 minutes in each position with the geko™ T-1 device switched off and then repeated after the geko™ T-1 device has been active for 10 minutes.

6. A new neuromuscular device to improve hemodynamic stability in critical care patients

The study is at the final planning stage, it has been designed to investigate the potential use of the neuromuscular device in stabilising the vascular systems of patients under critical care. Such patients are at an increased risk of developing DVT. The aim of the study is to create and clinically evaluate a medical device employing OnPulse™ technology, which is believed to improve cardiovascular status of critical care patients whilst at the same time prevent DVT. This will significantly reduce the number of deaths in critical care patients and reduce the average time of stay in hospitals. The objective of the study is to develop a modified OnPulse™ device designed for critical care patients by demonstrating its efficacy on patients under general anaesthesia. Another objective is to investigate the systemic hemodynamic benefits of the novel technology, particularly at and soon after induction of general anaesthesia.

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Appendix 1 - Ethics Approval Letter for Cardiac Study


National Research Ethics Service
Brent Medical Ethics Committee

Room 019, Level 7 Maternity Block
 Northwick Park Hospital
 Watford Road
 Harrow
 Middlesex
 HA1 3UJ

Tel: 020 8869 3805
 Fax: 020 8869 5222

08 January 2009

Dr Arthur Tucker
 Clinical Scientist & Senior Lecturer
 4th Floor Dominion House
 St. Bartholomew's Hospital
 London
 EC1A 7BE

Dear Dr Tucker

Study title: A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects
REC reference: 05/Q0408/14
Protocol number:
EudraCT number: Not applicable
Amendment number:
Amendment date: 12 December 2008

The above amendment was reviewed at the meeting of the Sub-Committee of the REC held on 07 January 2009.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Protocol	1.7.0	12 December 2008
Participant Information Sheet: Cardiac sub-group	1.0	12 December 2008
Covering Letter		12 December 2008
Annex 2 Notification of Amendment (CTIMPs)		12 December 2008

Membership of the Committee

The members of the Committee who were present at the meeting are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
 The National Research Ethics Service (NRES) represents the NRES Directorate within
 the National Patient Safety Agency and Research Ethics Committees in England

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

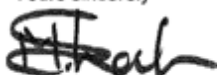
The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/Q0408/14:

Please quote this number on all correspondence

Yours sincerely



Mrs Mona Shah
Committee Co-ordinator

E-mail: Mona.Shah@nwlh.nhs.uk

Enclosures

List of names and professions of members who were present at the meeting and those who submitted written comments

Copy to:

Clinical Trials Unit, MHRA

Mr Gerry Leonard
Barts and The London NHS Trust
Joint Research Office, 3rd Floor
Rutland House, 42-46 New Road,
The Royal London
Hospital, Whitechapel, London E1 1BE

Appendix 2- Subject Information Sheet for Cardiac Study

A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects

Lay Title: “THRIVE-1 – Cardiac Sub-Group”

Part 1

Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Queen Mary, University of London (QMUL) is the sponsor of this research.

What is the purpose of the study?

You are being asked to take part in this study because you are a healthy adult, aged between 18 and 65 years.

Deep vein thrombosis (DVT) is the formation of a thrombus (clot) within the deep veins. It is a relatively common condition in patients receiving medical or surgical treatment in hospital. Recently there has been much controversy and media interest regarding the development of deep vein thrombosis and its effects in passengers undertaking long haul air travel. This is also referred to as the ‘economy class syndrome’ or ‘travellers thrombosis’ as it has also been associated with other modes of travel e.g. coach and car travel

The THRIVE-1 study is examining a novel method for increasing blood flow in the lower leg using low level electrical stimulation. Increases in leg blood flow improve the return of blood towards the heart and thereby reducing accumulation of blood in the legs (venous stasis). Since stasis is one of the important factors in the development of deep vein thrombosis, the risk of developing thrombosis should be reduced. This technique may prove to be a useful tool in preventing and reducing the incidence of deep vein thrombosis in both medical and surgical

patients together with passengers undertaking long duration travel (as travel-related DVT is not exclusively related to flying).

Following completion the first phase of 30 health Volunteers, we have found that the device can significantly increase leg blood flow in the arteries, veins and small blood vessels of the legs. The increases appear to be clinically valuable in relation to rest, but within normal ranges compared to exercise. Furthermore, there were no adverse events or causes for concern.

This sub-study will allow us to study if these increases in blood flow in the legs have the effect of increasing the output of the heart in 10 healthy Volunteers. The value of this additional study will be to further confirm the safety of the device and to understand if the THRIVE system may have the additional potential to help patients with heart disease.

Why have I been chosen?

You are being asked to take part in this study because you are a healthy adult, aged between 18 and 65 years.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. If you are employed by Barts & The London NHS Trust or Barts & The London School of Medicine & Dentistry, non-participation or dropping out of the study will not affect your training or career.

Before you can begin the study

You may read the full study protocol as well as this Information sheet, which gives you many details about the study. The recruiting Investigator will tell you about any potential adverse events that could occur in this study. You will be told exactly what the study entails and what will be required of you. You are encouraged to ask questions of the Investigators conducting the recruitment interview until you are satisfied that you fully understand the nature of the study and the requirements.

What will happen to me if I take part?

Screening

If you agree to take part and sign a ***Consent for Study Screening*** you will be invited to a selection session. We will take a detailed medical history and a physical examination, including blood pressure, pulse rate and heart rhythm. Signs of drug and alcohol abuse will also be checked. We will use an ultrasound machine to look at the blood vessels in your legs and your heart to check for any abnormalities, which might prevent you joining the study. This is entirely painless, and you will be required to attend with a pair of shorts.

Study

If all results match our inclusion and exclusion criteria you will be invited to join the study and you will be asked to start within 7 days. The duration of your participation in the study will be around 2 hours in total.

We hope to recruit 10 people to this part of the study. On the day of the study you will need to attend the Cardiac Department of the Royal London Hospital with a pair of shorts to wear during the testing. You will be instructed to have a light breakfast, avoiding fatty foods, tobacco and caffeine and will be asked to abstain from vigorous exercise from the previous evening onwards. You will be asked to rest for 30m minutes to relax and get use to the environment. Your resting blood flow will then be measured in your legs and an ultrasound study of your heart will occur. These measurements are entirely non-invasive and painless.

We will apply the stimulator electrodes to the back of each of your knees over a nerve. When switched on, the stimulation will make the muscles of your calf and foot gently contract. This will not hurt, but might feel a little strange and uncomfortable. The device will be turned on for the next 30 minutes, during which time the blood flow and heart measurements will be repeated. Also, during this time, we will ask you a series of questions about how the device feels and how comfortable you are compared to the discomfort you associate with having your blood pressure measured.

After this point you will be discharged from the Study.

Expenses and payments

For attending the Screening session we will compensate you with £15-00. At the end of the study we will be pleased to pay you £60-00 for your time, commitment to the study and expenses (a total maximum payment of £75-00).

If the study is stopped by the Investigator(s) prior to completion, if you withdraw or are withdrawn from the study before completion, a pro-rata payment will be made at the discretion of the Investigator(s).

What are the possible benefits of taking part?

This study will not benefit you directly, however it may lead to the development of a novel method for preventing deep vein thrombosis in patients and travellers alike; and may help in the future management of patients with heart disease

Could I come to any harm if I take part in the study?

You may be withdrawn from the study if the doctors feel it is best for you or if you do not comply with the requirements of the study.

If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel unacceptable discomfort, or for any reason during the study you do not wish to continue, than we will stop the tests immediately.

The blood flow measurements and ultrasound are non-invasive, painless and known to be entirely safe.

All of the previous work using the system was found to be safe. When the device is applying an electrical stimulation, you may feel some muscle twitching and maybe tingling in your lower legs.

There are very few risks involved in using this type of equipment and the device is commonly used for therapeutic purposes to exercise muscles under the supervision of a Physiotherapist, as well as by members of the public for “toning” purposes in their own homes.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

If you have a complaint please contact the following in the first instance: Dr. Arthur Tucker.

If you feel any discomfort or distress during the investigations, you must say so and we will stop the tests immediately at any time.

If you are employed by Barts & The London NHS Trust or Barts & The London School of Medicine & Dentistry, non-participation or dropping out of the study will not affect your training or career.

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

A contact number for complaints will be given.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

If you require any further information please contact:

Dr Arthur Tucker

The Ernest Cooke Vascular & Microvascular Unit,
4th Floor Dominion House,
St. Bartholomew's Hospital,
West Smithfield.
London. EC1A 7BE.
Tel Number: 0207 601 8498
This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will need to use the data collected up to your withdrawal.

What if there is a problem?

Queen Mary University of London has agreed that if you are harmed as a result of your participation in the study, you will be compensated, provided that, on the balance of probabilities, an injury was caused as a direct result of the intervention or procedures you received during the course of the study. These special compensation arrangements apply where an injury is caused to you that would not have occurred if you were not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

Complaints:

If you have a concern about any aspect of this study, you should ask to speak with the Research Team who will do their best to answer your questions (Dr Arthur Tucker, telephone 020 7601 8498).

If you remain unhappy and wish to complain formally you can do this by contacting: The Complaints Officer, c/o The Chief Operating Officer for the Barts and The London, Queen Mary School of Medicine and Dentistry, Wardens Office, 32 Newark Street, Whitechapel, London E1 2AA, 020 7882 2259

Will my taking part in this study be kept confidential?

All the information obtained about you in the course of the study is confidential and will be kept in a secure locked room. The investigators performing the study and a study Monitor will have access to the data collected in this study. They may also be looked at by representatives of regulatory authorities and by authorised

people from Queen Mary University of London Medical School to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

Involvement of the General Practitioner/Family doctor (GP)

With your permission we will contact your General Practitioner to advise them of your potential participation in this study.

What will happen to the results of the research study?

The results of this study may be published or presented at meetings. You will not be identified in any report / publication or presentation.

Who is organising and funding the research?

This research is being sponsored by Queen Mary, University of London Medical School.

Who has reviewed the study?

This study has been given a favourable ethical opinion for conduct in the NHS by Brent Research Ethics Committee reference 05 / Q0408 / 14.

Before you sign this consent form please ask any questions you have about the study.

Thank you for taking the time to read this information sheet.

Appendix 3- Screening Consent Form for Cardiac Study

WRITTEN CONSENT FORM:

REC Number: 05 /Q0408 / 14

ver 1.0

Title of research proposal: A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects – *Cardiac Sub-Group*
(Protocol number: THRIVE-1)

Name of Researcher: Dr Arthur T. Tucker

Name of Patient / Volunteer (Block Capitals):

Address:

SCREENING CONSENT FORM

(Initial boxes to agree with statement)

1. I confirm that I have read and understand the THRIVE-1 – Cardiac Sub-Group information sheet **dated 12th December 2008 version 1.0**, for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
1. I understand that my participation of the **SCREENING** is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
2. I understand that relevant sections of any of my data generated during the study may be looked at by responsible individuals from Queen Mary University of London Medical School and from regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
3. I agree to my GP being informed of my participation in the study and subsequently informed any significant information relevant to my participation. ☐
4. I agree to take part in the above study. ☐

 Name of Patient

 Date

 Signature

 Name of Person taking consent
 (if different from researcher)

 Date

 Signature

 Researcher

 Date

 Signature

When completed, 1 for Research Subject and 1 for researcher site file (original)

Appendix 3- Consent form for Study Participation for Cardiac Study

WRITTEN CONSENT FORM:

REC Number: 05 /Q0408 / 14

ver 1.0

Title of research proposal: A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects – *Cardiac Sub-Group*
(Protocol number: THRIVE-1)

Name of Researcher: Dr Arthur T. Tucker

Name of Patient / Volunteer (Block Capitals):

Address:

STUDY CONSENT FORM

(Initial boxes to agree with statement)

2. I confirm that I have read and understand the THRIVE-1 – Cardiac Sub-Group information sheet **dated 12th December 2008 version 1.0**, for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
1. I understand that my participation of the **STUDY** is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
2. I understand that relevant sections of any of my data generated during the study may be looked at by responsible individuals from Queen Mary University of London Medical School and from regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
3. I agree to my GP being informed of my participation in the study and subsequently informed any significant information relevant to my participation. ☐
4. I agree to take part in the above study. ☐

 Name of Patient

 Date

 Signature

 Name of Person taking consent
 (if different from researcher)

 Date

 Signature

 Researcher

 Date

 Signature

When completed, 1 for Research Subject and 1 for researcher site file (original)

Appendix 4- Ethics Approval Letter for THRIVE Study



National Research Ethics Service

Brent Medical Ethics Committee

Room 007, Level 5, L Block
Northwick Park Hospital
Watford Road
Harrow
Middlesex
HA1 3UJ

Tel: 020 8869 3805
Fax: 020 8869 5222

30 January 2008

Dr Arthur Tucker
Clinical Scientist & Senior Lecturer
4th Floor Dominion House
St. Bartholomew's Hospital
London
EC1A 7BE

Dear Dr Tucker

Study title: A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects
REC reference: 05/Q0408/14
Protocol number:
EudraCT number: Not applicable
Amendment number: By letter
Amendment date: 16 January 2008

Thank you for your letter of 16 January 2008, notifying the Committee of the above amendment.

It is noted that you do not consider this to be a substantial amendment to the clinical trial authorisation, as defined in the Medicines for Human Use (Clinical Trials) Regulations 2004, and that ethical review by the Committee is therefore not required.

The committee reviewed the documents at the meeting on 28 January 2008 and has approved the completion date to be extended to 31 July 2008.

Documents received

The documents received were as follows:

Document	Version	Date
Insurance Certificate-Sky Medical Technology		03 January 2008
Notification of a Minor Amendment	By letter	16 January 2008
Letter of Sponsorship-Queen Mary University of London		10 January 2008

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/Q0408/14:

Please quote this number on all correspondence

Yours sincerely



Mrs Mona Shah
Committee Co-ordinator

E-mail: Mona.Shah@nwh.nhs.uk

Copy to:

Mr Gerry Leonard
Barts and The London NHS Trust
Joint Research Office, 3rd Floor
Rutland House, 42-46 New Road,
The Royal London
Hospital, Whitechapel, London E1 1BE
E1 1BE

Appendix 5: Subject Information Sheet for THRIVE Study

A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects

EAST LONDON & The CITY HEALTH AUTHORITY

Invitation to participate in a Research Project

We invite you to take part in a research project, which we think may be important. The information, which follows, tells you about it. It is important that you understand what is in this leaflet. It says what will happen if you take part and what the risks might be. Try to make sure you know what will happen to you if you decide to take part. Whether or not you do take part is entirely your choice. Please ask any questions you want to about the research and we will try our best to answer them.

Introduction

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relative and your GP if you wish. Ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Consumers for Ethics in Research (CERES) publish a leaflet entitled "Medical Research and You". This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from the Research Team.

Why have we approached you?

You are being asked to take part in this study because you are a healthy adult, aged between 18 and 65 years.

Deep vein thrombosis (DVT) is the formation of a thrombus (clot) within the deep veins. It is a relatively common condition in patients receiving medical or surgical treatment in hospital. Recently there has been much controversy and media interest regarding the development of deep vein thrombosis and its effects in passengers undertaking long haul air travel. This is also referred to as the 'economy class syndrome' or 'travellers thrombosis' as it has also been associated with other modes of travel e.g. coach and car travel.

The THRIVE-1 study will examine a novel method for increasing blood flow in the lower leg using electrical stimulation. Increases in leg blood flow improve the return of blood towards the heart and thereby reducing accumulation of blood in the legs (venous stasis). Since stasis is one of the important factors in the development of deep vein thrombosis, the risk of developing thrombosis should be reduced. This technique may prove to be a useful tool in preventing and reducing the incidence of deep vein thrombosis in both medical and surgical patients together with passengers undertaking long duration travel (as travel-related DVT is not exclusively related to flying).

The aim of this second phase of the study is to effectiveness and tolerability of an electrical stimulation protocol developed in the first phase of investigations.

Before you can begin the study

You may read the full study protocol as well as this Information sheet, which gives you many details about the study. The recruiting Investigator will tell you about any potential adverse events that could occur in this study. You will be told exactly what the study entails and what will be required of you. You are encouraged to ask questions of the Investigators conducting the recruitment interview until you are satisfied that you fully understand the nature of the study and the requirements.

What would I do in the study, if I took part?

Screening

If you agree to take part and sign a ***Consent for Study Screening*** you will be invited to a selection session. We will take a detailed medical history and a physical examination, including blood pressure, pulse rate and heart rhythm. Signs of drug and alcohol abuse will also be checked. We will use an ultrasound machine to look at the blood vessels in your legs to check for any abnormalities, which might prevent you joining the study. This is entirely painless, and you will be required to attend with a pair of shorts.

With your permission we will contact your General Practitioner to advise them of your potential participation in this study.

Study

If all results match our inclusion and exclusion criteria you will be invited to join the study and you will be asked to start and complete your participation within a 2-day period.

We hope to recruit 20 people to this part of the study. On the day of the study you will need to attend the Clinical Microvascular Unit with a pair of shorts to wear during the testing. You will be instructed to have a light breakfast, avoiding

fatty foods, tobacco and caffeine and will be asked to abstain from vigorous exercise from the previous evening onwards. Before we start the blood flow measurements, we will insert 3 cannulae: one in a vein in each foot and one in your arm. By using the cannula we will be able to take small samples of blood without having to keep using a needle each time. At regular intervals: At the start, 1, 2, 3, and 4 hours we will take blood samples, twenty millilitres (four teaspoons) from each site at each time point. The total amount of blood we will collect for analysis is about 240mL, which is less than you would donate at the blood bank.

You will sit in an airline chair for a period of 4 hours. After sitting comfortably, we will ask you to move your toes up and down ten times to give us a baseline measurement of your natural system for moving the blood in the legs. Following this baseline, we will apply the stimulator electrode to the back of one of your knees over a nerve. When switched on, the stimulation will make the muscles of your calf and foot contract. This will not hurt, but might feel a little strange. Your other leg will not be stimulated and we will compare the responses of both legs. After each 5 minutes stimulation sequence we will take repeated measurements of the blood flow in your legs. Your blood flow will then be allowed to recover before the next sequence. These measurements are entirely non-invasive and painless. Also, during the 4 hours, we will ask you a series of questions about how the device feels and how comfortable you are compared to the discomfort you associate with having your blood pressure measured. During the study we will provide you with soft drinks (no alcohol) and a light snack. We hope to offer an 'in flight' movie, but you may prefer to work or read quietly.

After the 4-hour assessment period, we will rescan your legs with an ultrasound to check that there is no evidence of significant clotting. Our experience strongly indicates that this is not a significant risk to you.

After this point you will be discharged from the Study.

What payment will I receive for doing this study?

For attending the Screening session we will compensate you with £25-00. At the end of the 4-hour flight we will be pleased to pay you £90-00 for your time and a further £10-00 for each additional ultrasound for your time, commitment to the study and expenses (a total maximum payment of £135-00).

If the study is stopped by the Investigator(s) prior to completion, if you withdraw or are withdrawn from the study before completion, a pro-rata payment will be made at the discretion of the Investigator(s).

Will this study help me?

This study will not benefit you directly; however it may lead to the development of a novel method for preventing deep vein thrombosis in patients and travellers alike.

Could I come to any harm if I take part in the study?

You may be withdrawn from the study if the doctors feel it is best for you or if you do not comply with the requirements of the study.

If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel unacceptable discomfort, or for any reason during the study you do not wish to continue, then we will stop the tests immediately.

Previous work using the system was found to be safe. When the device is applying an electrical stimulation, you may feel some muscle twitching and maybe tingling in your lower leg.

There are very few risks involved in using this type of equipment and the device is commonly used for therapeutic purposes to exercise muscles under the supervision of a Physiotherapist, as well as by members of the public for “toning” purposes in their own homes.

There are very few risks involved in inserting a catheter into a vein in the arm. You may feel some discomfort and sometimes there may be a small bruise around the area, which may sore and last for a couple of days.

Due to the time you are sat in the chair, there is a very small statistical risk of microthrombi (clot) forming in your veins. The chance of this happening is similar to the risk accepted by any airline passenger. You have been selected as a group of people who should be at the lowest risk of developing a problem. However, the Volunteers will benefit from ultrasound monitoring by expert staff before and after each sitting session allowing early identification of any problems, and appropriate measures to be taken.

Symptoms of a clot can include: swelling of the leg, warmth and redness of the leg; pain that is noticeable; or worse when standing or walking; shortness of breath; chest pain which may be worsened by deep breaths; and coughing up phlegm, possibly flecked with blood.

Anyone with these symptoms should seek emergency medical treatment

Any concerns during the Study?

If you have any problems before, during or after the study you should contact:

Dr Arthur T. Tucker (020) 76018498. Outside working hours you can contact us via the hospital switchboard (0207) 3777000. The study has been seen and approved by the Brent Medical Research Ethics committee. Any personal information will remain strictly confidential.

If you feel any discomfort or distress during the investigations, you must say so and we will stop the tests immediately at any time.

If you are employed by Barts & The London NHS Trust or Barts & The London School of Medicine & Dentistry, non-participation or dropping out of the study will not affect your training or career.

Are there any factors, which would exclude me from taking part in the research? (And which are not known by the investigators) e.g. Other medications.

You may not join the study if you have ever had a deep vein thrombosis, significant injury to either of your legs or have any circulatory problems.

We need to know whether you are taking **any** medication as this may also exclude you from the study, this includes oral contraceptives and the so-called “morning-after pill”. There are no known interactions with any other medications but you must inform one of the Investigators of any new medications or changes in your general health during the study.

You should not take part in this study if you are already involved in any other study.

You will also not be able to take part in the study if you are taking *drugs of abuse*, either short or long term.

How will confidentiality be protected?

All the information obtained about you in the course of the study is confidential and will be kept in a secure locked room.

The investigators performing the study, Queen Mary University of London (study Sponsor) and the Barts & The London NHS Trust will have full access to the data collected in this study. Official representatives of the Drug Regulatory Authorities may at some stage in the future request access to the data collected in this study.

You don't have to join the study. You are free to decide not to be in this trial or to drop out at any time.

What happens if you are worried or if there is an emergency?

You will always be able to contact an investigator to discuss your concerns and/or to get help

Dr Arthur T. Tucker

The Ernest Cooke Clinical Microvascular Unit,
4th Floor Dominion House,
St. Bartholomew's Hospital,
London. EC1A 7BE.
Tel. No: **020 76018498**
Mobile No: **07887 852995**

What happens if something goes wrong?

We believe that this study is basically safe and do not expect you to suffer any harm or injury because of your participation in it. However, we carry insurance to make sure that if your health does suffer as a result of your being in the study, then you will be compensated. In such a situation, you will not have to prove that the harm or injury, which affects you, is anyone's fault. If you are not happy with any proposed compensation, you may have to pursue your claim through legal action.

Appendix 6 -Screening Consent Form for THRIVE Study

PRIVACY INFORMATION AND INFORMED CONSENT FORM FOR SCREENING

REC Number: 05 / Q0408 / 14

Title of research proposal: **A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects (Protocol number: THRIVE-1)**

Name of Patient / Volunteer (Block Capitals):

Address:

- The study organisers have invited me to take part in this research. ☐
- I understand what is in the leaflet about the research. I have a copy of the Patient's Information leaflet version 1.1.1 to keep. ☐
- I have had the chance to talk and ask questions about the study. ☐
- I agree to participate in the recruitment procedures for the above clinical study ☐
- I agree to providing such personal particulars and details of my medical/personal history as are necessary for the Research Team to assess my suitability for inclusion in the study. ☐
- I agree to have routine screening tests performed, covering medical history, a physical examination, measurement of the blood vessels in my legs, and assessment of any signs of drug or alcohol abuse ☐
- If accepted into the Investigation, I know what my part will be and I know how long it will take. ☐
- I know that an Independent Research Ethics Committee has seen and agreed this study. ☐
- I understand that my personal information is strictly confidential: I know the only people who may see information about my part in the study are the research team or an official representatives of the organisation, which Sponsored the research. ☐
- I freely consent to be a subject in the study. No-one has put pressure on me. ☐
- I know that I can stop taking part in the study at any time. ☐
- I know that the Investigators will contact my General Practitioner regarding my involvement. ☐
- I know that if I am employed or associated with Barts & The London NHS Trust or the Medical School, my involvement in this Study will have no bearing on my employment or studies. ☐
- I know that if there are any problems, I can contact: ☐

Dr Arthur T. Tucker

The Ernest Cooke Clinical Microvascular Unit,
4th Floor Dominion House,
St. Bartholomew's Hospital,
London. EC1A 7BE.
Tel. No: **020 76018498**
Mobile No.: **07887 852994**

Volunteer's Signature: Date:

Time: (24-hour)

Witness's Name:

Witness's Signature:

PRIVACY INFORMATION AND INFORMED CONSENT FORM FOR SCREENING

The following should be signed by the Investigator responsible for obtaining consent

As the Investigator responsible for this research or a designated deputy, I confirm that I have explained to the Volunteer named above the nature and purpose of the research to be undertaken.

Investigator's Name:

Investigator's Signature: Date:

Appendix 7 – Consent Form for Participation in THRIVE Study

INFORMED CONSENT FORM FOR STUDY PARTICIPATION

WRITTEN CONSENT FORM: REC Number: 05 / Q0408 / 14 ver 1.1.1 - 10th July 2005

Title of research proposal: **A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects (Protocol number: THRIVE-1)**

Name of Patient / Volunteer (Block Capitals):

Address:

- The study organisers have invited me to take part in this research. ☐
- I understand what is in the leaflet about the research. I have a copy of the Patient's Information leaflet version 1.1.1 to keep. ☐
- I have had the chance to talk and ask questions about the study. ☐
- I know what my part will be in the study and I know how long it will take. ☐
- I know how the study may affect me. I have been told if there are possible risks. ☐
- I understand that I should not take part in more than one study at a time. ☐
- I know that an Independent Research Ethics Committee has seen and agreed this study. ☐
- I understand that personal information is strictly confidential: I know the only people who may see information about my part in the study are the research team or an official representative of the organisation which Sponsored the research. ☐
- I freely consent to be a subject in the study. No-one has put pressure on me. ☐
- I know that I can stop taking part in the study at any time. ☐
- I know that if I am employed or associated with Barts & The London NHS Trust or the Medical School, my involvement in this Study will have no bearing on my employment or studies. ☐
- I know that if there are any problems, I can contact: ☐

Dr Arthur T. Tucker

The Ernest Cooke Clinical Microvascular Unit,
4th Floor Dominion House,
St. Bartholomew's Hospital,
London. EC1A 7BE.
Tel. No: **020 76018498**
Mobile No.: **07887 852994**

Volunteer's Signature: Date:

Time: (24-hour)

Witness's Name:

Witness's Signature:

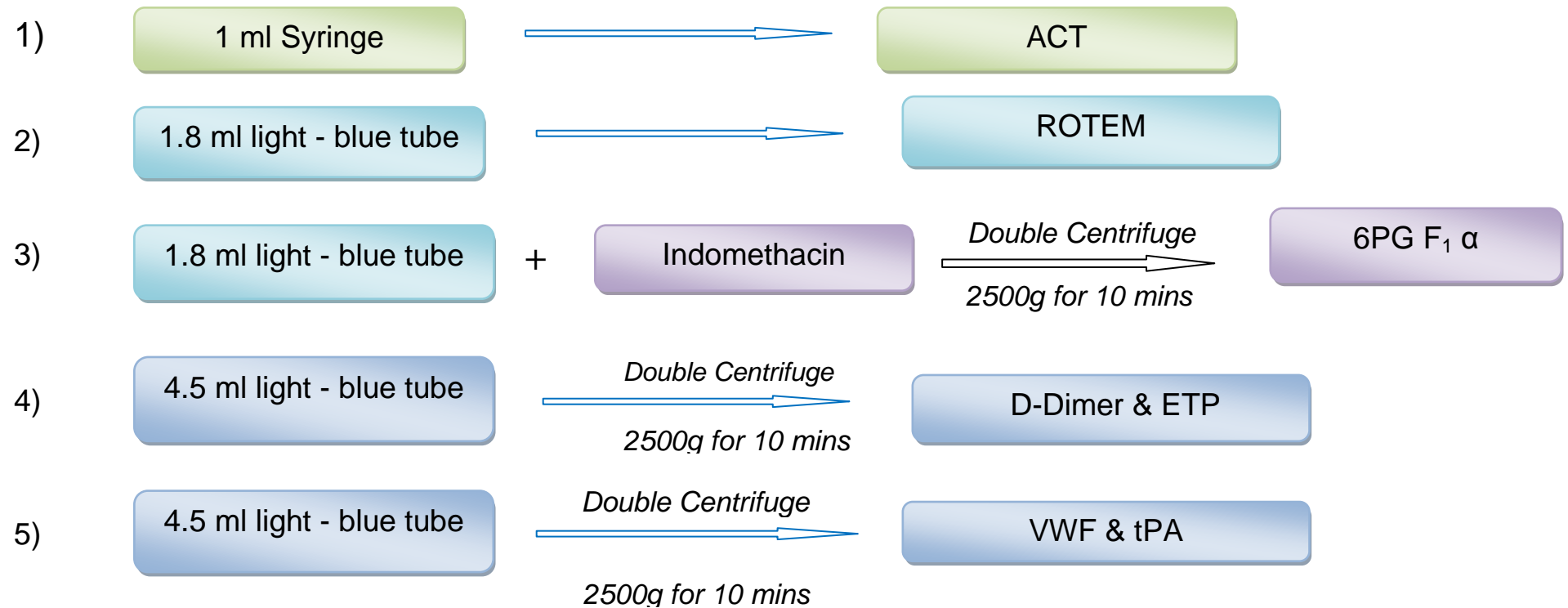
The following should be signed by the Investigator responsible for obtaining consent
As the Investigator responsible for this research or a designated deputy, I confirm that I have explained to the Volunteer named above the nature and purpose of the research to be undertaken.

Investigator's Name:

Investigator's Signature: Date:

Appendix 8- Flow Chart for Blood Collection

Blood sample taken from [a] Left foot [b] Right foot [c] Arm of each subject at 0,1,2,3, and 4 hours goes to:



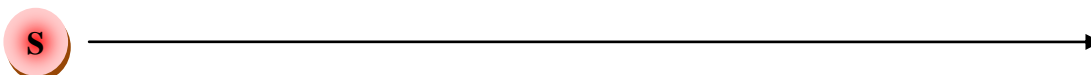
Appendix 9-Cryo-vial Arrangement Plan per Patient

A1: Start of D-Dimer & ETP (0 - 4 hrs)**G2:** Start of PGF1 alpha (0 - 4 hrs)**G7:** Start of vwf & Tpa (0 - 4hrs)

Arm (A)

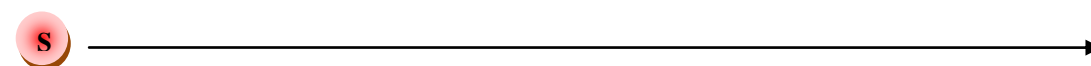
Right Leg (RL)

Left Leg (LL)



Box 1

A1 0hr (A)	B1 1hr	C1 2hr	D1 3hr	E1 4hr	F1 0h (RL)	G1 1hr	H1 2hr	I1 3hr
A2 4hr	B2 0hr (LL)	C2 1hr	D2 2hr	E2 3hr	F2 4hr	G2 0hr (A)	H2 0hr	I2 0hr
A3 1hr	B3 1hr	C3 1hr	D3 2hr	E3 2hr	F3 2hr	G3 3hr	H3 3hr	I3 3hr
A4 4hr	B4 4hr	C4 4hr	D4 0hr (RL)	E4 0hr	F4 0hr	G4 1hr	H4 1hr	I4 1hr
A5 2hr	B5 2hr	C5 2hr	D5 3hr	E5 3hr	F5 3hr	G5 4hr	H5 4hr	I5 4hr
A6 0hr (LL)	B6 0hr	C6 0hr	D6 1hr	E6 1hr	F6 1hr	G6 2hr	H6 2hr	I6 2hr
A7 3hr	B7 3hr	C7 3hr	D7 4hr	E7 4hr	F7 4hr	G7 0hr (A)	H7 0hr	I7 0hr
A8 0hr	B8 0hr	C8 0hr	D8 0hr	E8 1hr	F8 1hr	G8 1hr	H8 1hr	I8 1hr
A9 1hr	B9 1hr	C9 2hr	D9 2hr	E9 2hr	F9 2hr	G9 2hr	H9 2hr	I9 2hr



Box 2

A1 3hr	B1 3hr	C1 3hr	D1 3hr	E1 3hr	F1 3hr	G1 3hr	H1 4hr	I1 4hr
A2 4hr	B2 4hr	C2 4hr	D2 4hr	E2 4hr	F2 0hr (RL)	G2 0hr	H2 0hr	I2 0hr
A3 0hr	B3 0hr	C3 0hr	D3 1hr	E3 1hr	F3 1hr	G3 1hr	H3 1hr	I3 1hr
A4 1hr	B4 2hr	C4 2hr	D4 2hr	E4 2hr	F4 2hr	G4 2hr	H4 2hr	I4 3hr
A5 3hr	B5 3hr	C5 3hr	D5 3hr	E5 3hr	F5 3hr	G5 4hr	H5 4hr	I5 4hr
A6 4hr	B6 4hr	C6 4hr	D6 4hr	E6 0hr (LL)	F6 0hr	G6 0hr	H6 0hr	I6 0hr
A7 0hr	B7 0hr	C7 1hr	D7 1hr	E7 1hr	F7 1hr	G7 1hr	H7 1hr	I7 1hr
A8 2hr	B8 2hr	C8 2hr	D8 2hr	E8 2hr	F8 2hr	G8 2hr	H8 3hr	I8 3hr
A9 3hr	B9 3hr	C9 3hr	D9 3hr	E9 3hr	F9 4hr	G9 4hr	H9 4hr	I9 4hr

Appendix 10- Standard Operating Instructions for ACT



Operating Instructions for the Medtronic Hemotec ACT II machine.

Low Range ACT Tests

- 1) Connect power cable and turn on at rear of machine. The machine automatically runs through a self-check test. The temperature displayed should read between 36 – 37 °C (body temperature) this initially takes about 10 mins.

Do **not** turn on the Incubation switch. This option is not required for Low Range ACT tests.

- 2) **Place the test cartridges in the machine** for **3 minutes** prior to collecting sample of blood. This step will heat the cartridges up to 36-37 C (body temp.). These cartridges can be left heating in the machine for up to 12 hours.
- 3) **Collect your blood sample** when ready to perform test. Approx. **0.5 ml required**.
- 4) **Tap the test cartridges** to mix the reagents in lower reagent chamber.
- 5) When using Low Range test cartridges (product code 402-01) Inject **.2ml** of blood sample into each upper reaction chamber of test cartridge, this should fill to **between the fill lines**. Allow the sample to flow down the back of the chamber taking care to avoid getting sample on the black flag. (we recommend using a syringe with blunt tip needle to dispense the sample in the cartridge).
- 6) **Insert the test cartridge into the machine** (actuator heat block) and **push forward to closed position**. The test will commence immediately.

After clot formation has been detected, an audible tone sounds and the actuator heat block automatically springs forward to open position. The result is displayed (in seconds) on the front panel, until the next test is commenced. If wished - new cartridges can be inserted to prepare for next test.

The results have two display formats controlled by the front panel **Display** switch. When the display switch is depressed so that “**Channel 1**” and “**Channel 2**” are illuminated, the individual clotting times are displayed.

Channel 1 or the left –hand channel is the upper display, and channel 2 or the right-hand Channel is the lower display. When the Display switch is depressed so that “**Average**” and “**Difference**” are illuminated, the upper display shows the average of the clotting times of channels 1 and 2, and the bottom display shows the difference between the clotting times of channels 1 and 2.

NB: The Duplicate channel results should fall within 12% of each other for extended or heparinised blood samples. If the values do not fall within 12% of each other please retake the test with a fresh blood sample.

Appendix 11- Standard Operating Procedures for Rotem®

> Performing A Test



1. properly attach pin



2. insert cup and bring to position using the MC rod



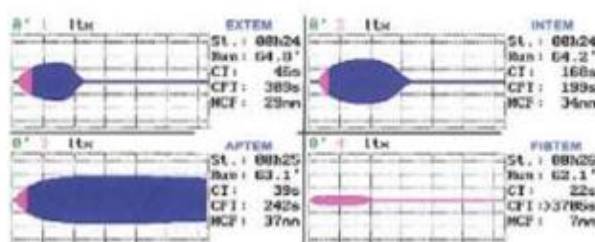
3. select test



8. discard used cup and pin

EXTEM: Extrinsically activated TEM (Tissue Factor activation)
 —> Confirm each step by pressing the pipette's ENTER button
 —> Always press the button briefly
 Put the pipette tip INTO the star-TEM reagent (green) —> ENTER
 Take the tip OUT of the reagent —> ENTER (for air cushion)
 Put the tip INTO the ex-TEM reagent (red) —> ENTER
 Hold the tip OVER the cup —> ENTER
 Put a NEW tip INTO the citrated blood sample —> ENTER
 Hold the tip OVER the cup —> ENTER
 Put the tip to the bottom of the cup —> ENTER (for mixing)
 Hold the tip OVER the cup —> ENTER

4. pipetting steps are displayed on the screen



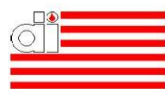
7. on screen display of TEMograms and numeric parameters



5. pipetting of reagents and blood



6. insert cup holder in measurement position after mixing of reagents and sample



american diagnostica inc.

500 West Avenue, Stamford, CT 06902
Tel. (203) 602-7777 • Fax. (203) 602-2221



IMUBIND® tPA ELISA

REF 860



8°C
2°

CPT Code 85400

EC REP

american diagnostica GmbH
Kaplanelgasse 35, D-64319 Pfungstadt, Germany
Tel. + 49 61 57 / 99 08 99 • Fax + 49 61 57 / 99 08 08

ENGLISH

INTENDED USE

The IMUBIND® tPA ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative measurement of human tissue-type Plasminogen Activator (tPA) antigen in plasma. This kit is for *in vitro* diagnostic use.

EXPLANATION OF THE TEST

Tissue-type Plasminogen Activator (tPA) was first identified as a key component of the fibrinolytic system in 1940 having been found in various tissues and organs.^{1,2} The role of tPA is to activate plasminogen to plasmin, which in turn degrades fibrin resulting in thrombolysis. Native tPA exists as a single-chain molecule or as a proteolytically degraded two-chain molecule consisting of 527 amino acids with a molecular ratio (M) of 70,000 D.³ While the single-chain molecule possesses less activity to various substrates⁴, both forms of tPA are equally active toward plasminogen.⁵ tPA is unique in this aspect as it has an enzymatic activity even in its single-chain form. Plasma tPA is also known to be complexed with serpin Plasminogen Activator Inhibitor Type-1 (PAI-1).

Localized to endothelial cells in arterial vessel walls, tPA is released into the blood stream upon various stimuli such as venous occlusion, physical exertion, and stress, particularly in atherosclerotic patients.⁶ Plasma tPA levels may be affected as a result of drug interactions^{7,8,9,10} and may be reduced as a result of cigarette smoking.^{11,12} Levels of tPA in human plasma are also known to be elevated during late stage pregnancy.^{13,14} Furthermore, studies have shown that plasma levels of endogenous tPA may be useful as a risk marker for myocardial infarction^{15,16} and stroke.^{17,18}

PRINCIPLE OF THE METHOD

The IMUBIND tPA ELISA Kit uses microwells coated with a goat polyclonal antibody directed against human tPA and containing soluble non-immune goat antibody. tPA standards and plasma samples are added to the wells and the antibody captures the tPA antigen present in the plasma during an incubation period. Then peroxidase conjugated goat polyclonal antibody against human tPA (same as the coating antibody) is added to the well. The antibody conjugate binds to the captured tPA molecules. Next, all unbound material is washed away and then a peroxidase reactive enzyme substrate, ortho-phenylenediamine (OPD) is added to the wells. The subsequent peroxidase/substrate reaction yields a yellow colored solution. Addition of sulfuric acid stops the reaction and turns the solution color orange. The absorbance of the solution is measured at 490 nm. The absorbance is directly proportional to the amount of tPA present in the sample.

REAGENTS

- R1 tPA Microplate, 6 strips of antibody coated microwells, 16 wells per strip, in a frame with 1 Acetate Sheet to cover the microwell frame during assays
- R2 1 vial PET Buffer (PBS-EDTA-Tween 20, powder), 15.18 g
- R3 3 vials of tPA Plasma Standard, 30 ng/mL (lyophilized)
- R4 3 vials of tPA Depleted Plasma Standard, 0 ng/mL (lyophilized)
- R5 1 vial Detection Antibody Conjugate, 100 µL (concentrate)
- R6 1 vial Substrate, OPD (lyophilized)
- R7 1 vial 0.15% Hydrogen Peroxide (H₂O₂), 2 mL

*There is a sufficient amount of reagents to assay 44 plasma samples and to generate a four-point standard curve (both tested in duplicate) for 96 tests. Samples may be patient, control, or reference plasmas.

WARNING

The source material for reagents in this kit have been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using US Food and Drug Administration approved methods. As no known test method can provide complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious human specimen.

Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards (OPD is toxic). Avoid splashing or aerosol formation. Do not expose the OPD reagent to strong sunlight. Store OPD in the dark and do not allow it to come into contact with any oxidizing agents or metal parts.





R45/R20/R21/R22/R36/R37/R38/R42/
R43/S45/S26/S36/S37/S39*

MATERIALS REQUIRED BUT NOT PROVIDED

0.22 micron filtered deionised water
 8-channel pipette covering 50 - 200 µL
 1-channel pipettes covering 10 - 1000 µL
 repetitive pipette 50 µL
 squeeze bottle
 towels or thin sponge
 50 mL containers, e.g., plastic tubes
 microwell plate washer, microwell plate orbital shaker
 microwell plate reader set at a wavelength of 490 nm
 concentrated sulfuric acid (H₂SO₄)
 centrifuge (capable of 1,500 x g)



REAGENT PREPARATION AND STORAGE**A. PET Buffer (R2)**

Dissolve the contents of the PET Buffer vial in 1 L of filtered deionised water. Mix for 15 minutes. Prepared buffer may be stored for:

	1 month
	2° to 8°C

B. tPA Standards (R3, R4)

- To the tPA Plasma Standard, 30 ng/mL, and the tPA Depleted Plasma Standard, 0 ng/mL, add 0.5 mL filtered deionised water. Agitate gently for 3 minutes. Treat the standards as you would a plasma sample (see SPECIMEN COLLECTION AND PREPARATION). Standards should be used within one hour after reconstitution. Reconstituted standards may also be aliquotted into tightly capped tubes and stored for:

	6 months
	-70°C or colder

- Prepare a 10 ng/mL standard and a 20 ng/mL standard for the calibration curve by adding the 30 ng/mL tPA Plasma Standard and the 0 ng/mL tPA Depleted Plasma Standard in the following ratios. Mix each tube for a few seconds. Pipette the 30 ng/mL and 0 ng/mL standards directly from their vials.

tPA Standard	Volume of 30 ng/mL tPA Plasma Standard	Volume of 0 ng/mL tPA Depleted Plasma Standard
10 ng/mL	50 µL	100 µL
20 ng/mL	100 µL	50 µL

C. Detection Antibody Conjugate (R5)

Prepare working strength Detection Antibody Conjugate by diluting 1:100 with PET Buffer. For running all 96 microwells at one time, dilute 60 µL of Detection Antibody Conjugate up to 6 mL in PET Buffer. If all 96 microwells are not to be used, dilute 10 µL of Detection Antibody Conjugate up to 1 mL in PET Buffer for each 16 microwell strip that will be used. Working strength Detection Antibody Conjugate may be stored in the dark for:

	1 month
	-20°C

D. Substrate (R6)

Add 2 mL of filtered deionised water to the vial and gently agitate for five minutes to prepare concentrated substrate. Store prepared substrate at:

	1 month
	-20°C

The substrate may be thawed and refrozen. Prepare normal strength OPD substrate within 30 minutes of use in the assay by mixing 300 µL of substrate concentrate with 300 µL of hydrogen peroxide and 1.5 mL of filtered deionised water for each 16 well strip used. (For using the entire plate, transfer the 2 mL of concentrated substrate to a larger glass tube and add an additional 10 mL of filtered deionised water. Then add the full contents (2 mL) of the hydrogen peroxide vial to prepare normal strength substrate.

E. Hydrogen Peroxide (R7)

After opening, store in the dark at:

	1 month
	2° to 8°C

F. Stop Solution

Prepare a 1.5 M H₂SO₄ solution by adding 2.5 mL of concentrated sulfuric acid (95 - 97%) to 27.5 mL of filtered deionised water. Mix well. Store unused Stop Solution in a glass bottle at:

	1 month
	20° to 25°C

SPECIMEN COLLECTION AND PREPARATION

Either citrate or EDTA collected platelet poor plasma may be used for this assay. See "Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays", NCCLS Document H21-A3, Vol. 18, No. 20, December 1999.²⁰ Plasma collection should be performed as follows:

- Collect 9 parts of blood into 1 part of 3.2% (0.109M) trisodium citrate anticoagulant solution.
- Centrifuge the blood sample at 1500 x g for 15 minutes.
- Plasma should be stored at 2° to 8°C and assayed within 2 hours. Alternatively, plasma may be stored for:

	2 weeks	6 months
	-20°C	-70°C

- Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2° to 8°C and assayed within 2 hours.

ASSAY PROCEDURE

- The six antibody coated microwell strips are provided assembled in a plastic holder, giving the appearance of a fixed 96-microwell plate. Please note that the strips are constructed such that they fit into the frame in only one orientation. Open the foil pouch and remove the strip/frame assembly. In the foil pouch is a packet of desiccant. Do not discard this desiccant. Keep the desiccant in the pouch for storing unused microwell strips. Determine the number of microwells needed to generate the standard curve and assay the number of unknown specimen samples and references/controls. Remember, it is recommended to assay standards and samples in duplicate. Remove the strips that will not be used, replace in the foil pouch and tightly reseal the pouch with the desiccant inside. Store the sealed pouch at 2° to 8°C until the expiration date printed on the pouch label.
- Add 50 µL of prepared PET Buffer to each microwell.
- Dilution of plasma samples is seldom necessary, but samples containing more than 30 ng/mL of tPA can be diluted with the 0 ng/mL tPA Depleted Plasma Standard or tPA/PAI Depleted Plasma (Amencan Diagnostica Inc. REF: 273). The amounts of tPA are then deduced from the calibration curve by multiplying the measured sample value by the dilution factor.
- Add 20 µL of tPA standard or plasma sample to each microwell. Cover the wells with the acetate sheet provided. Place the strip/frame assembly on an orbital microwell plate shaker with agitation (at 500-600 rpm) and incubate for one hour at room temperature (20° to 25°C).
- Remove the acetate cover sheet (do not discard) and add 50 µL of the Detection Antibody Conjugate to each microwell. **Do not empty the wells.** Replace the acetate sheet over the microwells and incubate on the plate shaker (at 500-600 rpm) for 15 minutes at room temperature (20° to 25°C).
- Remove the acetate sheet and empty the contents of the microwells. Wash the wells four times with the PET Buffer. Washing can either be performed using microwell plate washing equipment or manually (fill the wells with PET Buffer with a pipette or squeeze bottle, wait three minutes, empty and remove droplets by tapping the plate 4 to 5 times face down against absorbing material. Repeat four times.

7. Add 100 µL of Substrate to each microwell. Cover the wells and incubate on the plate shaker (at 500-600 rpm) for 15 minutes at room temperature (20° to 25°C).
 8. Add 50 µL of Stop Solution to each well. Add in the same order and at the same speed as the Substrate was added. Store the plate for 10 minutes in the dark to allow the color to stabilize.
- Caution!** The acid is corrosive and must be handled with care. Avoid skin and eye contact. Wear glasses and gloves.
9. Read the absorbance of the wells on a microwell plate reader at a wavelength of 490 nm within 30 minutes.

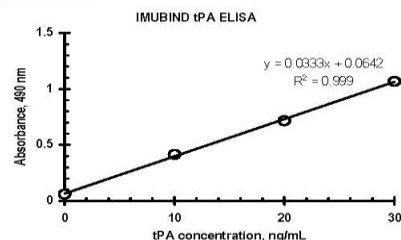
Procedural Note

Completely fill the microwells during the wash step and make sure the microwells are completely empty after each wash. Do not allow the microwells to dry out.

RESULTS

A standard curve is constructed by plotting the mean absorbance value measured for each tPA standard versus its corresponding tPA concentration. A standard curve should be constructed each time the assay is performed. The following curve is presented for demonstration purposes only.

Representative Standard Curve



CALCULATIONS

Interpolate the tPA concentration of the plasma sample directly from the standard curve. A curve regression software function that is included with the microwell plate reader may be used to calculate the concentrations.

Plasma samples found to contain more than 30 ng/mL tPA should be diluted with the 0 ng/mL tPA Depleted Plasma Standard supplied or tPA-PAI Depleted Plasma (American Diagnostica Inc. REF 273). These samples may be reassayed. The tPA concentration of the diluted sample interpolated from the standard curve is then calculated by multiplying the measured sample value by the dilution factor.

TRACEABILITY OF CONTROL MATERIALS

Information regarding traceability of control materials is available upon request from American Diagnostica Inc.²¹

QUALITY CONTROL

It is recommended that 2 plasma samples containing between 4-10 ng/mL tPA antigen (low control) and 12-20 ng/mL (high control) be stored at -20°C or below, in small aliquots and used as quality control standards each time the assay is run. Failure to obtain a tPA antigen level within 2 standard deviations of the mean for each control standard may invalidate the assay. The 30 ng/mL standard and the tPA Depleted Plasma are provided to construct the standard curve and must not be used to check device performance.

LIMITATIONS OF PROCEDURE

Sodium azide present in a test sample will inhibit the peroxidase activity of the conjugate antibody. If azide was present in the sample, wash the well after the sample incubation and refill the well with 70 µL PET buffer before adding the conjugate.

Bacterial contamination in the filtered deionised water may cause aberrant results.

INTERFERENCES

The presence of heparin (5.0 IU/mL), urokinase (100 IU/mL), or streptokinase (300 IU/mL) does not influence the determination of tPA antigen in plasma.

EXPECTED VALUES

In a group of 195 healthy adults, 95% of plasma tPA values were less than 9.0 ng/mL. The table below summarizes results for males, females and the combined group.

Group	Number of Donors	Median tPA (ng/mL)	95 th percentile tPA (ng/mL)
Females	95	3.9	8.3
Males	100	4.7	9.1
All	195	4.2	9.0

Plasma tPA levels may be affected as a result of drug interactions^{2, 9,9,10} and may be reduced as a result of cigarette smoking.^{11,12} Levels of tPA in human plasma are also known to be elevated during late stage pregnancy.^{13,14} Furthermore, studies have shown that plasma levels of endogenous tPA may be useful as a risk marker for myocardial infarction^{15,16} and stroke.^{17,18} Individual laboratories should determine normal ranges of tPA antigen levels using their local population. Studies have found that the level of tPA antigen increases with age.^{21,22}

PERFORMANCE CHARACTERISTICS

Precision

Studies evaluated the intra-assay and inter-assay variations of two (2) sample concentrations assayed in duplicate over 10 runs (n=20). The following variations were found.

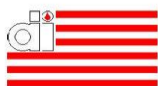
Sample	Mean Assayed tPA Value	Intra-Assay Variation	Inter-Assay Variation
No. 1	6.5 ng/mL	4.9%	8.2%
No. 2	14.9 ng/mL	4.2%	4.0%

Sensitivity

The assay quantifies various forms of human tPA antigen. It measures both the single-chain and two-chain forms of the molecule. Complexes of tPA/PAI-1 were found to read equivalent to tPA antigen. The lower limit of detection is 1 ng/mL tPA in the undiluted sample when the assay is performed according to the procedure.²³

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IMUBIND® vWF ELISA Kit

Product No. 828

INTENDED USE

The IMUBIND® vWF ELISA kit is an enzyme-linked immunoassay for the quantitation of vWF antigen in human plasma.

This assay is for research use only. It is not intended for diagnostic or therapeutic procedures.

EXPLANATION OF THE TEST

Von Willebrand Factor (vWF) is a large, multimeric protein (molecular weight of 1,000-20,000 kD) composed of repeating 270 kD subunits containing 2050 amino acid residues. vWF is synthesized by endothelial cells and megakaryocytes, and is present in multimeric form in the basement membrane of the subendothelium, in plasma and platelets. The half-life of vWF in plasma is approximately 20 hours. Degraded forms of vWF are excreted in urine.^{1,2} vWF functions as a carrier protein for Factor VIII, the coagulation protein absent in haemophilia A. It promotes platelet adhesion to damaged endothelium and participates in the platelet to platelet cohesion necessary for thrombus formation. Together with fibronectin and collagen, vWF functions in maintaining vessel wall integrity. Since vWF is synthesized in endothelial cells, it has been used as a marker for endothelial cell function and integrity. Measurements of vWF have been applied in a large number of basic investigations on endothelial cell function.

Patients with severe von Willebrand disorder (classified as type III) suffer from a complete absence of vWF in their plasma and urine. Patients with decreased circulating levels of vWF suffer from milder forms of the disorder, classified as type I, type IIa and type IIb.^{2,3} vWD type I is a common disorder⁴.

Studies have reported that in large vessel lesions of the endothelium such as arteriosclerosis, vWF levels have increased. However, in other studies, no significant increase of the factor has been observed⁵. Increased levels of the vWF/Factor VIII complex have also been reported in postoperative patients and patients with thrombosis⁶.

It has been demonstrated that the determination of the plasma level of vWF:Ag is of value in the diagnosis, prognosis and monitoring of therapy in systemic vasculitides and also for detecting latent vasculopathy^{7,8}. Therefore, monitoring vWF levels may be important in selecting those patients with systemic arthritis who require early cytotoxic therapy. In addition, high levels of vWF have been shown to be an indicator of poor prognosis in patients with septicemia⁹ and is considered to be a valuable marker of distant organ injury in patients having suffered skin burns.

vWF levels were measured in plasma and urine samples from patients with Type I diabetes mellitus (insulin-dependent) with and without signs of microangiopathy

(retinopathy and nephropathy)⁵. Plasma vWF levels were significantly higher in all groups of patients compared to control patients, while urinary vWF levels were significantly higher in patients with microangiopathy. Qualitative differences in the excreted forms of vWF were observed only in those patients with clinical signs of nephropathy, where there was a shift towards excretion of the high molecular weight fragments.

PRINCIPLE OF THE PROCEDURE

The IMUBIND vWF ELISA is a "sandwich" ELISA using a goat polyclonal antibody as the capture antibody. Samples incubate in precoated micro-test wells and the same polyclonal antibody, horseradish peroxidase (HRP) conjugated, is used to detect the bound vWF antigen. The addition of perborate/3,3',5,5' - tetramethylbenzidine (TMB) substrate, and its subsequent reaction with the HRP, creates a blue colored solution. Sensitivity is enhanced by the addition of a 0.5M sulfuric acid stop solution, yielding a yellow colored solution. vWF levels are determined by measuring and comparing the absorbance of sample solutions at 450 nm against those of a standard curve developed using calibrated antigen.

REAGENTS

6 strips of 16 antibody coated microwells in holder and acetate cover sheet
6 vials vWF standards, 0 - 10 mU/mL (lyophilized)
1 vial Detection Antibody, HRP-conjugated anti-human vWF (135 µL)
1 vial Detection Antibody Diluent (lyophilized)
1 vial Substrate, TMB (11 mL)
1 packet Wash Buffer, PBS with 0.05% Tween 20, pH 7.4

There are sufficient reagents to assay 42 plasma samples and generate a 6-point standard curve (both tested in duplicate). Samples may be patient, control or reference plasmas.

Warning

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Limited for research use only in the United States. For *in vitro* use only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

1. Precoated Microwell Strips

Once removed from the foil pouch, the microwell strips should be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.

2. Standards

1. Add 1.0 mL distilled H₂O to the 0.5, 1.0, 2.0, 5.0 and 10.0 mU/mL standard vials.
2. Add 2.0 mL distilled H₂O to the 0 mU/mL standard vial.
3. Agitate gently. Do not shake!

Reconstituted vWF standards are stable for up to 1 month when stored at -20°C or colder.

3. Detection Antibody

Supplied as a concentrated solution. Detection Antibody is diluted to working strength immediately before adding to the microwells. Concentrated Detection Antibody is stable until the expiration date stated on the vial when stored at 2°-8°C. Unused working strength Detection Antibody should be discarded.

4. Detection Antibody Diluent

1. Add 20 mL of distilled H₂O to the Detection Antibody Diluent vial.
2. Mix well.

Reconstituted Detection Antibody Diluent may be used for up to 1 month when stored at 2°-8°C.

5. Substrate

Supplied ready to use. Once opened, the substrate may be used for up to 1 month when stored at 2°-8°C.

6. Wash Buffer

1. Dissolve the contents of the Wash Buffer packet in 900 mL distilled H₂O.
2. QS. to a final volume of 1 Liter with distilled H₂O.
3. Mix well and confirm pH is 7.4 (adjust if necessary).

Wash Buffer may be used for up to 1 month when stored at 2°-8°C.

7. Sample Buffer

Prepare an appropriate amount of Sample Buffer by adding BSA to Wash Buffer to a final concentration of 3% w/v (3 gm BSA/100 mL Wash Buffer). Sample Buffer may be used for up to 2 weeks when stored at 2°-8°C.

SPECIMEN COLLECTION AND PREPARATION

Either citrate or EDTA collected platelet poor plasma may be used for this assay. See "Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays", NCCLS Document H21-A3, Vol. 18, No. 20, December 1998. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 1,500 x g for 15 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at -20°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.
5. Dilute plasma sample 1:100 in Sample Buffer.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

0.22 µm filtered deionized H₂O

50-300 µL eight channel multi-pipette

0-200 µL, 200-1000 µL single pipettes

microwell plate reader for reading absorbance at 450 nm

microwell plate washer (optional)

0.5 M H₂SO₄ **Caution:** Handle Sulphuric acid with great care. Avoid any skin and eye contact.

Wear protection glasses and gloves when handling.

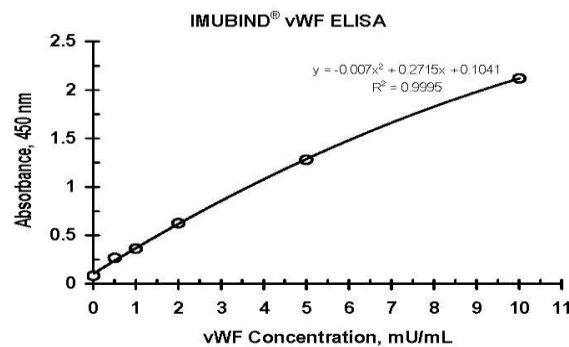
Bovine Serum Albumin (BSA, e.g. Serological)

1. Open the foil pouch and remove the microwell/frame assembly. Remove those microwell strips that will not be used and replace them in the foil pouch with the desiccant inside. Reseal the pouch and store at 2°-8°C.
2. Add 100 µL of vWF standard or diluted sample to a microwell, cover with the acetate sheet and incubate for 1 hour at room temperature. It is recommended to perform measurements in duplicate.
3. Wash the microwells 4 times with wash buffer (250 µL per well).
4. Prepare working strength Detection Antibody by diluting the concentrate 1:100 as follows: add 20 µL of Detection Antibody to 2 mL of Detection Antibody Diluent. Then add 100 µL of working strength Detection Antibody to each microwell, cover with the acetate sheet and incubate for 1 hour at room temperature.
5. Wash the microwells 4 times with wash buffer (250 µL per well).
6. Add 100 µL of Substrate to each microwell, cover with acetate sheet and incubate for 20 minutes at room temperature. A blue color will develop.
7. Stop the enzymatic reaction by adding 50 µL of 0.5M H₂SO₄. Tap the sides of the strip wells to ensure even distribution of the H₂SO₄. The solution color will turn yellow. Immediately read the absorbances of the solutions on a microwell plate reader set at a wavelength of 450 nm.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each vWF Standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

Representative Standard Curve



CALCULATION

Use the mean absorbance value for each diluted sample to interpolate its vWF concentration from the standard curve. Multiply the concentration determined from the standard curve by 100 (the dilution factor) to obtain the vWF concentration in the original plasma sample.

LIMITATIONS OF THE PROCEDURE

There are no known limitations for this assay.

PERFORMANCE CHARACTERISTICS

The performance characteristics of this assay have yet to be determined.

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6-keto-Prostaglandin $F_{1\alpha}$ ELISA Kit Instructions

Please read all instructions carefully before beginning this assay

PRODUCT #404310

For Research Use Only

*****Store kit at 4°C at all times*****

Do not freeze kit components

DESCRIPTION

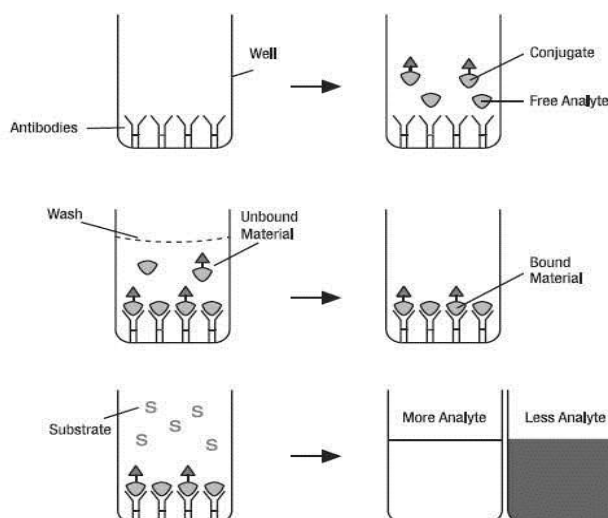
6-keto-Prostaglandin $F_{1\alpha}$ (6-k-PGF $_{1\alpha}$) is a stable hydrolyzed product of unstable PGI $_2$ (prostacyclin). PGI $_2$ is derived from PGH $_2$ which in turn is synthesized from the cyclooxygenase pathway. Through this pathway, it is the major product produced by the endothelial cells, although it is also synthesized in other cell types such as macrophages. Prostacyclin inhibits platelet aggregation and induces vasodilation. Quantitation of prostacyclin production can be made by determining the level of 6-k-PGF $_{1\alpha}$.

PRINCIPLE OF ASSAY

This is an ELISA (Enzyme-Linked Immunosorbent Assay) for the quantitative analysis of 6-keto-Prostaglandin $F_{1\alpha}$ levels in biological fluid. This test kit operates on the basis of competition between the enzyme conjugate and the 6-k-PGF $_{1\alpha}$ in the sample for a limited number of binding sites on the antibody coated plate.

The sample or standard solution is first added to the microplate. Next, the diluted enzyme conjugate is added and the mixture is shaken and incubated at room temperature for one hour. During the incubation, competition for binding sites is taking place. The plate is then washed removing all the unbound material. The bound enzyme conjugate is detected by the addition of substrate which generates an optimal color after 30 minutes. Quantitative test results may be obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a microplate reader at 650 nm. The extent of color development is inversely proportional to the amount of 6-k-PGF $_{1\alpha}$ in the sample or standard. For example, the absence of 6-k-PGF $_{1\alpha}$ in the sample will result in a bright blue color, whereas the presence of 6-k-PGF $_{1\alpha}$ will result in decreased or no color development.

PRINCIPLE OF ASSAY (continued)



MATERIALS PROVIDED

1. **EIA BUFFER:** 30 mL. Provided to dilute enzyme conjugate and 6-k-PGF_{1α} standards.
2. **WASH BUFFER (10X):** 20 mL. Dilute 10-fold with deionized water. This is used to wash all unbound enzyme conjugate, samples and standards from the plate after the one hour incubation.
3. **K-BLUE SUBSTRATE:** 20 mL. Stabilized 3,3', 5,5' Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (H₂O₂) in a single bottle. It is used to develop the color in the wells after they have been washed. **LIGHT SENSITIVE.** Keep refrigerated.
4. **EXTRACTION BUFFER (5X):** 30 mL. Dilute 5-fold with deionized water. This is used for diluting extracted and non-extracted samples.
5. **6-KETO-PROSTAGLANDIN F_{1α} ENZYME CONJUGATE:** 150 µL. 6-k-PGF_{1α} horseradish peroxidase concentrate. Blue capped vial.
6. **6-KETO-PROSTAGLANDIN F_{1α} STANDARD:** 100 µL. 6-k-PGF_{1α} standard at the concentration of 1 µg/mL. Green capped vial.
7. **6-KETO-PROSTAGLANDIN F_{1α} ANTIBODY-COATED MICROPLATE:** A 96 well MaxiSorp™ Nunc microplate with anti-6-k-PGF_{1α} rabbit antibody precoated on each well. The plate is ready for use as is. **DO NOT WASH!**

MATERIALS NEEDED BUT NOT PROVIDED

1. 300 mL deionized water for diluting wash buffer and extraction buffer.
 2. Precision pipettes that range from 10 µL-1000 µL and disposable tips.
- NOTE: If all or several strips are to be used at one time, it is suggested that a multichannel pipette be used.**
3. Clean test tubes used to dilute the standards and conjugate.
 4. Graduated cylinders to dilute and mix wash buffer and extraction buffer.
 5. Microplate reader with 650 nm filter.
 6. Plate cover or plastic film to cover plate during incubation.

OPTIONAL MATERIALS:

7. 1 N HCl or Neogen's Red Stop Solution.
 8. Microplate shaker.
- If performing an extraction on samples, the following will be required:
9. Methanol
 10. Methyl formate
 11. 0.1 M Sodium Phosphate buffer, pH 7.5
 12. C₁₈ Sep-Pak® column (Waters® Corporation)
 13. Petroleum ether
 14. Nitrogen gas
 15. Vortex
 16. Centrifuge

WARNINGS AND PRECAUTIONS

1. **DO NOT** use components beyond expiration date.
2. **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
3. **DO NOT** pipette reagents by mouth.
4. Always pour substrate out of the bottle into a clean test tube - **DO NOT** pipette out of the bottle. If the pipette is unclean, this could result in contamination of the substrate.
5. All specimens should be considered potentially infectious. Exercise proper handling precautions.
6. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
7. Use aseptic technique when opening and removing reagents from vials and bottles.
8. Keep plate covered except when adding reagents, washing or reading.
9. Kit components should be refrigerated at all times when not in use.

PROCEDURAL NOTES

1. It is not necessary to allow reagents to warm to room temperature before use.
2. Desiccant bag must remain in foil pouch with unused strips. Keep zip-lock pouch sealed when not in use to maintain a dry environment.
3. Always use new pipette tips for the buffer, enzyme conjugate, standards and samples.
4. Before pipetting a reagent, rinse the pipette tip three times with that reagent (i.e. fill the tip with the desired amount of reagent and dispense back into the same vial - repeat 2 times). Now the tip is properly rinsed and ready to dispense the reagent into your well or test tube.
5. When pipetting into the wells, **DO NOT** allow the pipette tip to touch the inside of the well, or any of the reagents already in the well. This can result in cross contamination.
6. Standards and samples should be assayed in duplicate.
7. To quantitate, always run samples alongside a standard curve. If testing a sample that is not extracted, standards should be diluted in the same type of medium being tested. This medium should be known to be negative.
8. Gently mix specimens and reagents before use. Avoid vigorous agitation.
9. When using only partial amounts of a kit, it is recommended to transfer the appropriate volume of each reagent to a clean vessel for repeated dispensing. This will reduce reagent contamination by repeated sampling from the original container.
10. The enzyme conjugate is most stable in its concentrated form. Dilute only the volume necessary for the amount of strips currently being used.
11. Before taking an absorbance reading, wipe the outside bottom of the wells with a lint-free wiper to remove dust and fingerprints.
12. Before opening the enzyme conjugate and standard vial, tap vial in an upright position to remove any liquid in the cap.

SAMPLE PREPARATION

This assay is non-species specific. Usually, urine and tissue culture supernatant can be assayed directly by diluting them with the diluted extraction buffer. Plasma and most other mediums will need to be extracted.

EXTRACTION OF 6-K-PGF_{1α}

1. Add 0.2 mL of methanol to 1 mL of biological fluid and vortex.
2. For tissue, homogenize it in 15% methanol in 0.1 M sodium phosphate buffer, pH 7.5 (100 mg in 1 mL methanol - buffer). Centrifuge the homogenate for five (5) minutes. Collect the supernatant in a clean tube.
3. Precondition the C₁₈Sep-Pak® column (Waters® Corporation) by washing the column with 2 mL of methanol followed by 2 mL of water.
4. Apply the above sample into the column and adjust the flow rate to 1 mL per minute.
5. Wash the column with 2 mL of 15% methanol in water followed by 2 mL of petroleum ether.
6. The Prostaglandin is eluted by 2 mL of methyl formate.
7. Evaporate methyl formate eluate with a stream of nitrogen gas.
8. Dissolve the residue with 1 mL of diluted extraction buffer and assay 50 µL in duplicates.
9. If the concentration is higher than the high range of the standard curve, the samples in #8 need to be further diluted and assayed.

Note: *Extraction buffer must be diluted 5-fold with deionized water before use. Any precipitant present must be brought into solution before dilution.*

TEST PROCEDURES

1. Prepare standards as follows:

Standard	Preparation
A	stock solution 1 µg/mL (provided in green capped vial)
B	take 20 µL of A, add to 980 µL of EIA buffer and mix=20 ng/mL
C	take 200 µL of B, add to 1.8 mL of EIA buffer and mix=2 ng/mL
D	take 200 µL of C, add to 1.8 mL of EIA buffer and mix=0.2 ng/mL

Continue standard preparation following Scheme I.

SCHEME I

Standards	ng/mL	EIA buffer (µL added)	C standard µL	D standard µL
S ₀	0	as is	-	-
S ₁	0.02	900	-	100
S ₂	0.04	800	-	200
S ₃	0.1	500	-	500
S ₄	0.2	-	-	as is
S ₅	0.4	800	200	-
S ₆	1	500	500	-
S ₇	2	-	as is	-

2. Determine the number of wells to be used.
3. Dilute the 6-k-PGF_{1α} enzyme conjugate. Add 1 μL of enzyme conjugate into 50 μL total volume of EIA buffer for each well assayed. For the whole plate, add 110 μL of the enzyme conjugate into 5.5 mL total volume of EIA buffer. Mix the solution thoroughly.
4. Add 50 μL of standards (S) or unknown (U) (some samples may require diluting) to the appropriate wells in duplicate.

See Scheme II for suggested template design.

5. Add 50 μL of the diluted enzyme conjugate to each well. Use 8-channel pipette or 12-channel pipette for rapid addition.
6. Mix by shaking plate gently. A microplate shaker may be used.
7. Cover plate with plastic film or plate cover and incubate at room temperature for one hour. **Note: Keep plate away from drafts and temperature fluctuations.**
8. Dilute concentrated wash buffer with deionized water (i.e. 20 mL of wash buffer plus 180 mL of deionized water). Mix thoroughly.
9. After incubation, dump out the contents of the plate. Tap out contents thoroughly on a clean lint-free towel.
10. Wash each well with 300 μL of the diluted wash buffer. Repeat for a total of three washings. An automated plate washer can be used, however, increase wash cycles from three to five.
11. Add 150 μL of substrate to each well. Use multichannel pipette for best results. Mix by shaking plate gently.
12. Incubate at room temperature for 30 minutes.
13. Gently shake plate before taking a reading to ensure uniform color throughout each well.
14. Plate is read in a microplate reader at 650 nm. If a dual wavelength is used, set W₁ at 650 nm and W₂ at 490 nm.
15. If accounting for substrate background, use 2 to 8 wells as blanks with only substrate in the wells (150 μL/well). Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

NOTE: Some microplate readers can be programmed to do these subtractions automatically when reading the plate. Consult your instrument manual.

OPTIONAL TEST PROCEDURES

16. Add 50-100 μL of 1 N HCl or Neogen's Red Stop Solution to each well to stop enzyme reaction.
17. Read plate at 450 nm, if 1N HCl solution was used. Read plate at 650 nm, if Neogen's Red Stop Solution was used.
18. Plot the standard curve and estimate the concentrations of the samples from the curve. See "CALCULATIONS."

Note: Absorbance readings will approximately double when stopped with acid. If absorbance readings are too high for measuring with your microplate reader, decrease the substrate incubation approximately 10 minutes but no more than 15 minutes.

SCHEME II

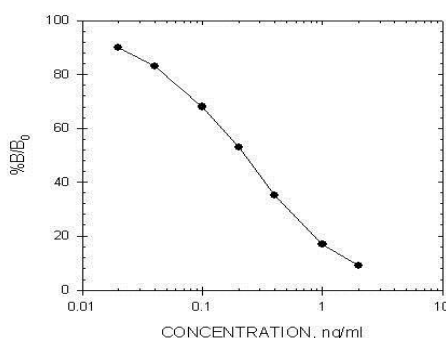
	1	2	3	4	5	6	7	8	9	10	11	12
A	S ₀	S ₀	U ₁	U ₁	U ₉	U ₉	U ₁₇	U ₁₇	U ₂₅	U ₂₅	U ₃₃	U ₃₃
B	S ₁	S ₁	U ₂	U ₂	U ₁₀	U ₁₀	U ₁₈	U ₁₈	U ₂₆	U ₂₆	U ₃₄	U ₃₄
C	S ₂	S ₂	U ₃	U ₃	U ₁₁	U ₁₁	U ₁₉	U ₁₉	U ₂₇	U ₂₇	U ₃₅	U ₃₅
D	S ₃	S ₃	U ₄	U ₄	U ₁₂	U ₁₂	U ₂₀	U ₂₀	U ₂₈	U ₂₈	U ₃₆	U ₃₆
E	S ₄	S ₄	U ₅	U ₅	U ₁₃	U ₁₃	U ₂₁	U ₂₁	U ₂₉	U ₂₉	U ₃₇	U ₃₇
F	S ₅	S ₅	U ₆	U ₆	U ₁₄	U ₁₄	U ₂₂	U ₂₂	U ₃₀	U ₃₀	U ₃₈	U ₃₈
G	S ₆	S ₆	U ₇	U ₇	U ₁₅	U ₁₅	U ₂₃	U ₂₃	U ₃₁	U ₃₁	U ₃₉	U ₃₉
H	S ₇	S ₇	U ₈	U ₈	U ₁₆	U ₁₆	U ₂₄	U ₂₄	U ₃₂	U ₃₂	U ₄₀	U ₄₀

CALCULATIONS

1. After the substrate background has been subtracted from all absorbance values, average all of your duplicate well absorbance values.
2. The average of your two S_0 values is now your B_0 value. (S_1 now becomes B_1 , etc.)
3. Next, find the percent of maximal binding ($\%B/B_0$ value). To do this, divide the averages of each standard absorbance value (now known as B_1 through B_7) by the B_0 absorbance value and multiply by 100 to achieve percentages.
4. Graph your standard curve by plotting the $\%B/B_0$ for each standard concentration on the ordinate (y) axis against concentration on the abscissa (x) axis. Draw a curve by using a curve fitting routine (i.e. 4-parameter or linear regression).
5. Divide the averages of each sample value by the B_0 value and multiply by 100 to achieve percentages.
6. Using the standard curve, the concentration of each sample can be determined by comparing the $\%B/B_0$ of each sample to the corresponding concentration of 6-k-PGF $_{1\alpha}$ standard.
7. If the samples were diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

6-keto-Prostaglandin $F_{1\alpha}$ in EIA Buffer



TYPICAL DATA

NOTE: "Typical data" is a representation. Variances in data will occur. Optical density readings may fluctuate during the shelf-life of the kit, but the $\%B/B_0$ should remain comparable. Measuring wavelength: 650 nm

Standard	Standard Concentration (ng/mL)	Optical Density (Absorbance Value)	$\%B/B_0$
S_0 (B_0)	0	1.223	100
S_1 (B_1)	0.02	1.101	90
S_2 (B_2)	0.04	1.014	83
S_3 (B_3)	0.1	0.835	68
S_4 (B_4)	0.2	0.645	53
S_5 (B_5)	0.4	0.428	35
S_6 (B_6)	1	0.210	17
S_7 (B_7)	2	0.110	9

Appendix 15 – Discomfort Questionnaire for THRIVE Study

Volunteer Number:

Time Point:

Date:

Discomfort Questionnaire

When compared to a blood pressure cuff inflated around your upper arm, how does the stimulation feel?

Q: “How uncomfortable was the last stimulation sequence?”

Select one answer by circling the number.

① ② ③ ④ ⑤

[1] No sensation (other than muscles tensing and relaxing)

[2] Minimal sensations

[3] Mild discomfort

[4] Moderate discomfort

[5] Severe discomfort

Fold Here ----- Fold Here

Q: “What did the last stimulation sequence feel like?”

Make a vertical line across the line below, which relates to the intensity of any discomfort experienced by the procedure.

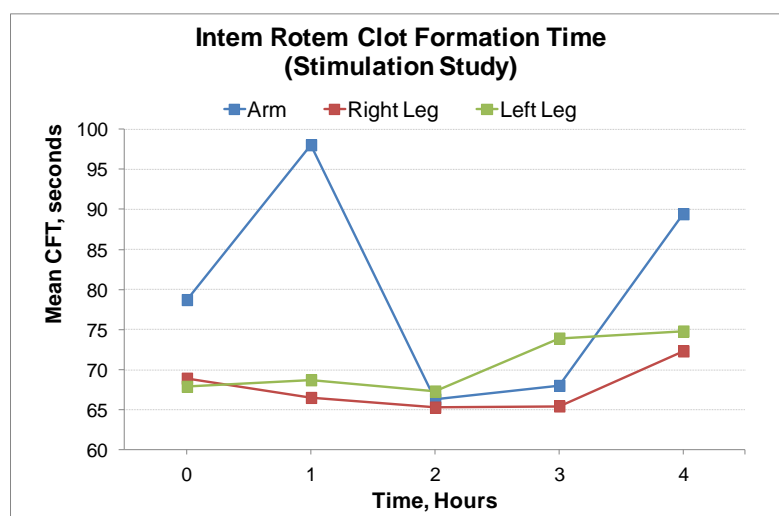
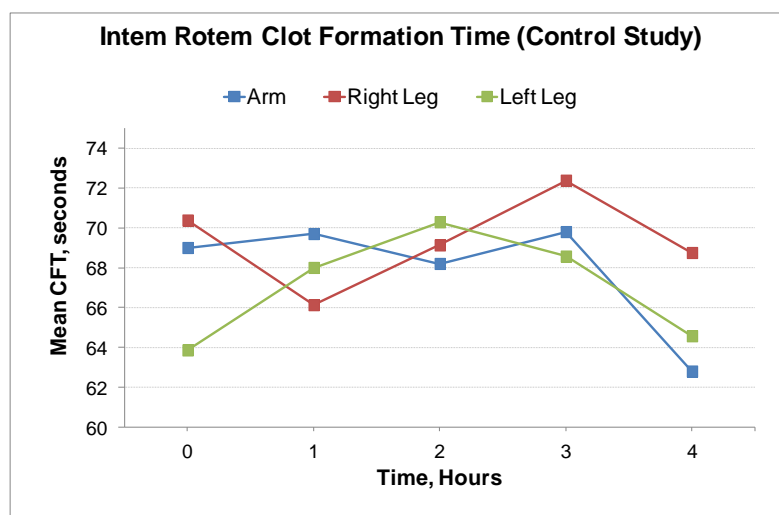
9.1.1.1.

No Sensations

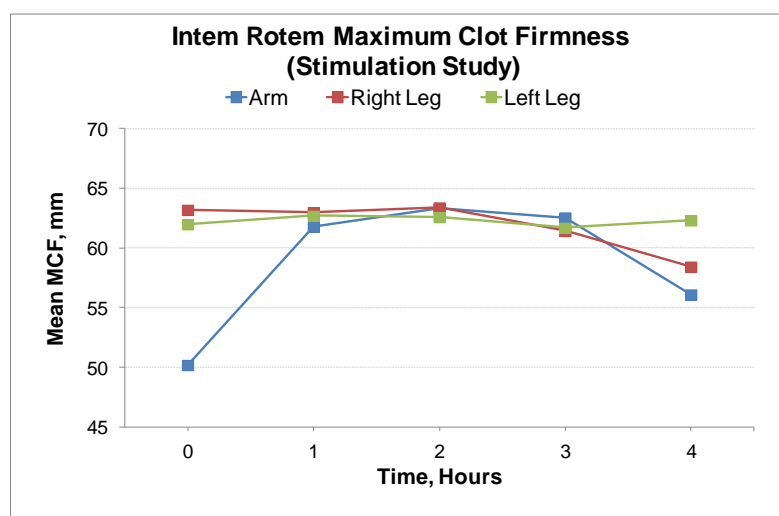
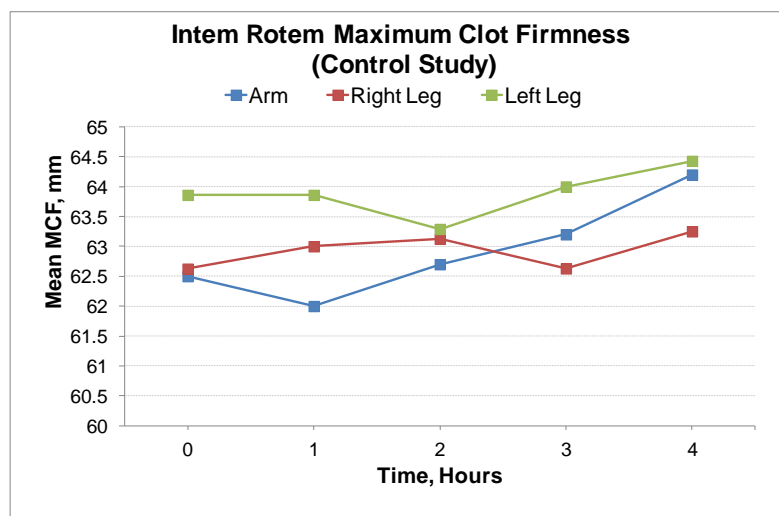
Severe Discomfort

Appendix 16 - Clotting Time Assessment using Rotem® Device

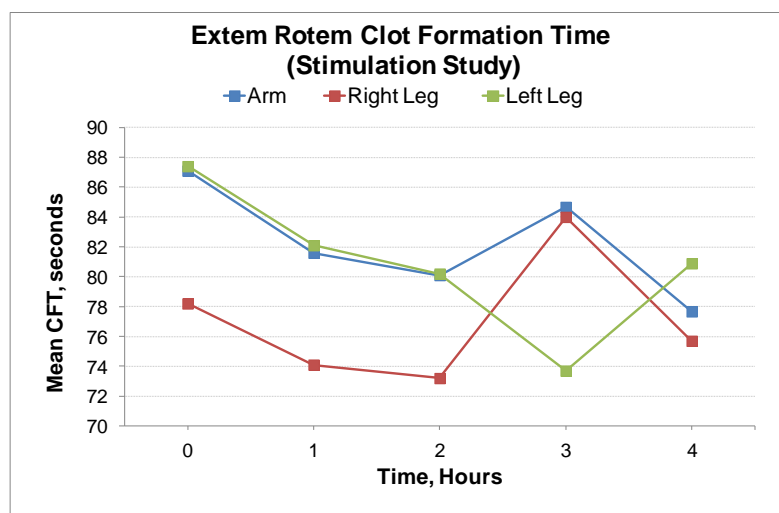
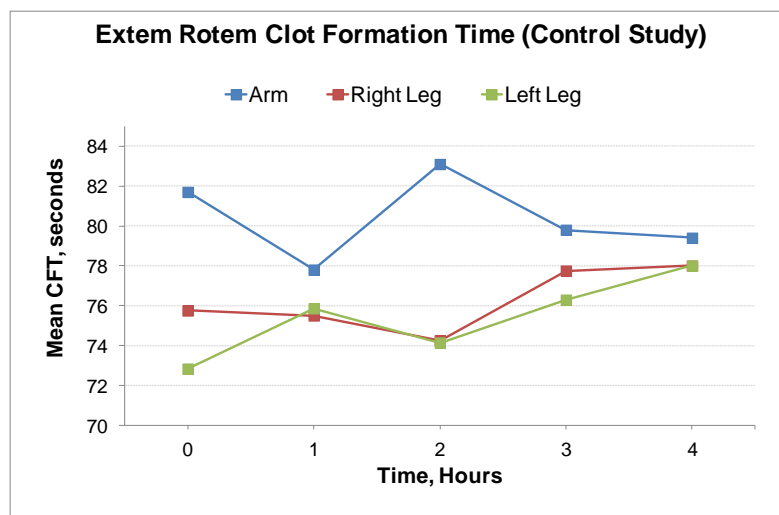
A. Clot formation time using Intem Rotem® throughout the control and Stimulation Study



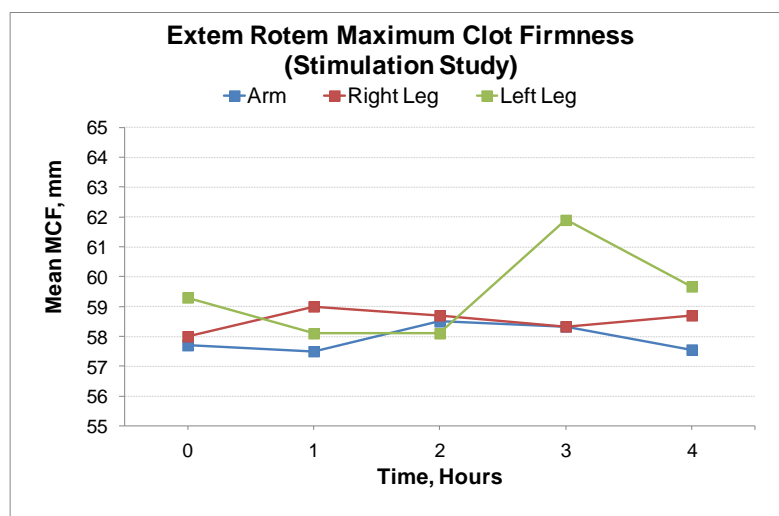
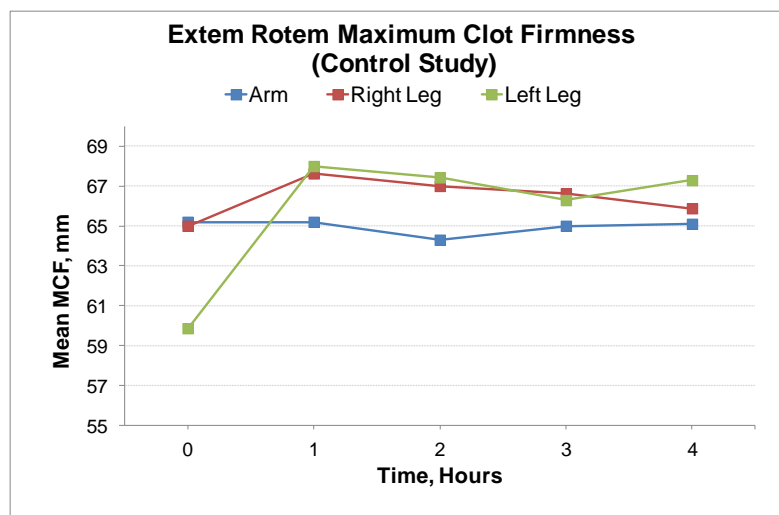
B. Maximum Clot Firmness using Intem Rotem® throughout the Control and Stimulation Study



C. Clot Formation Time using Extem Rotem® throughout the Control and Stimulation Study

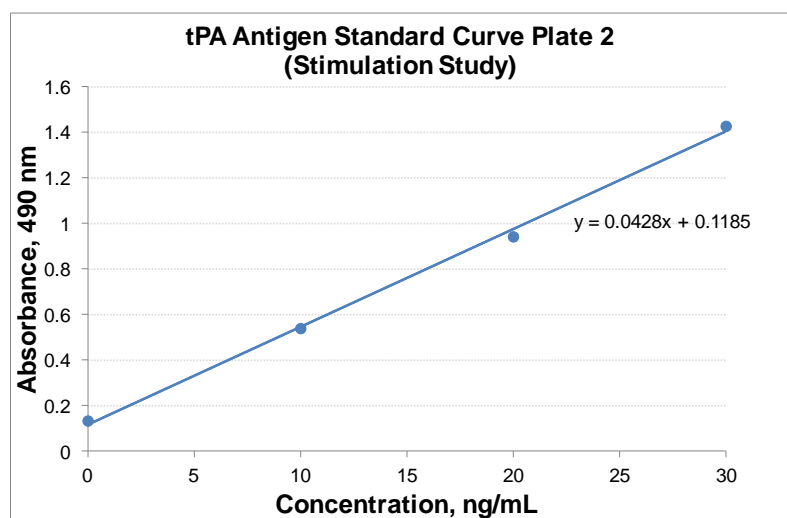
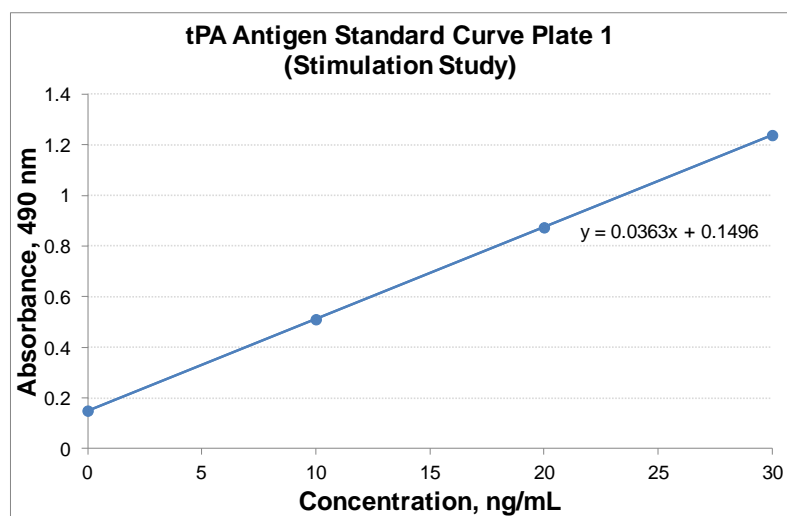


D. Maximum Clot Firmness using Exem Rotem® throughout the Control and Stimulation Study

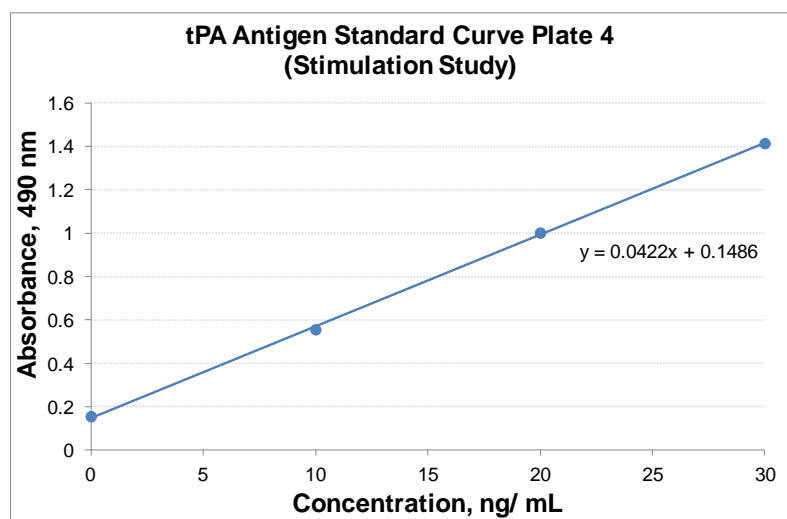
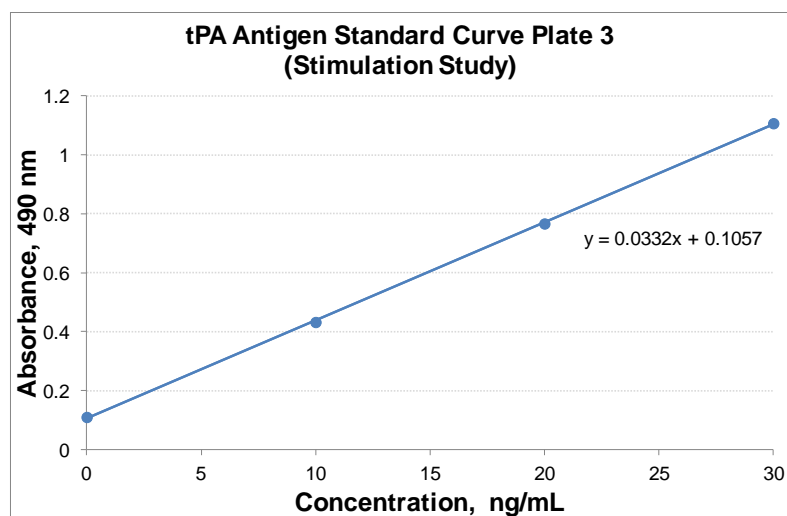


Appendix 17 - Tissue Plasminogen Activator Antigen Data

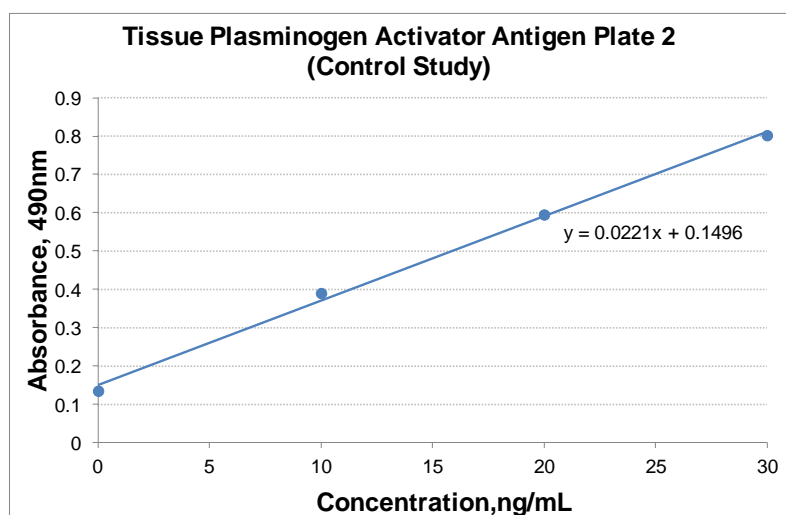
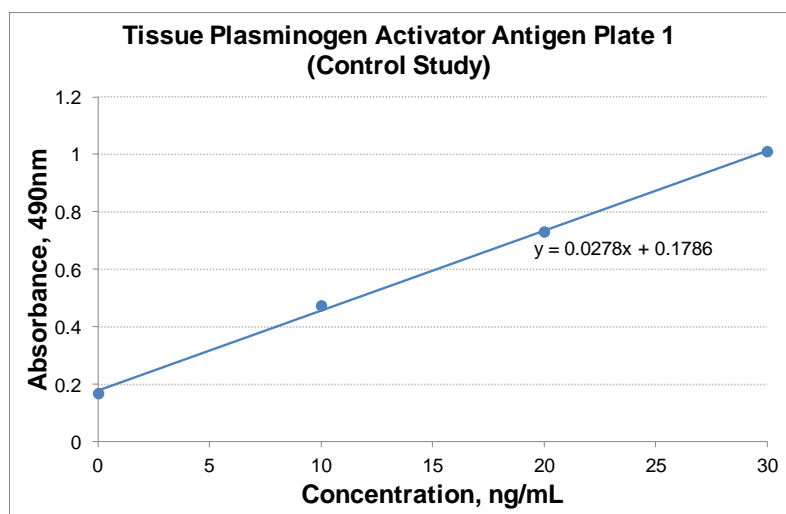
Standard Curves for Plates 1 & 2 (Stimulation Study)



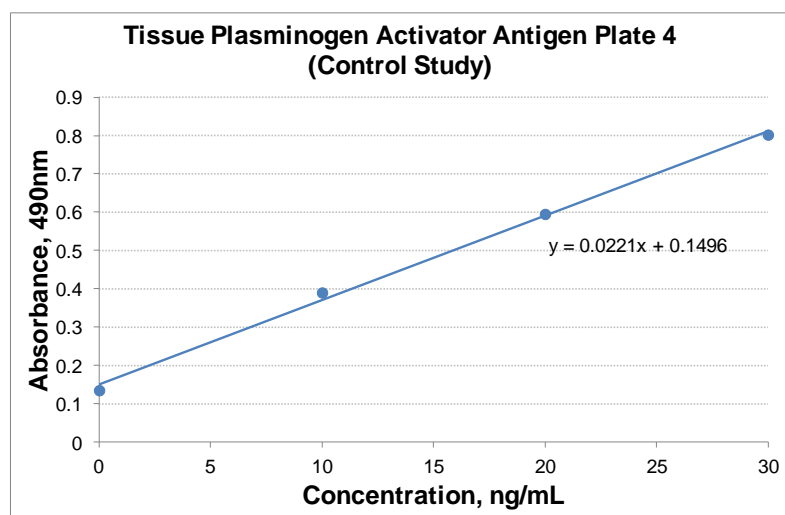
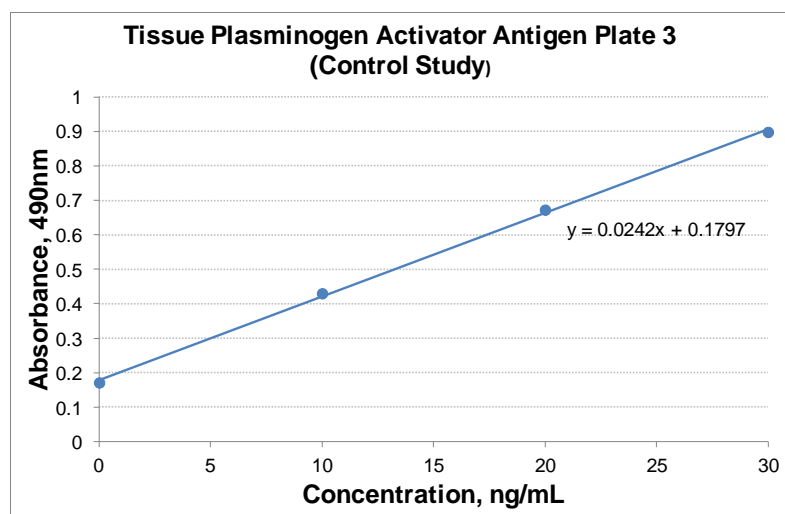
Standard Curves for Plates 3 & 4 (Stimulation Study)



Standard Curves for Plate 1 & 2 (Control Study)

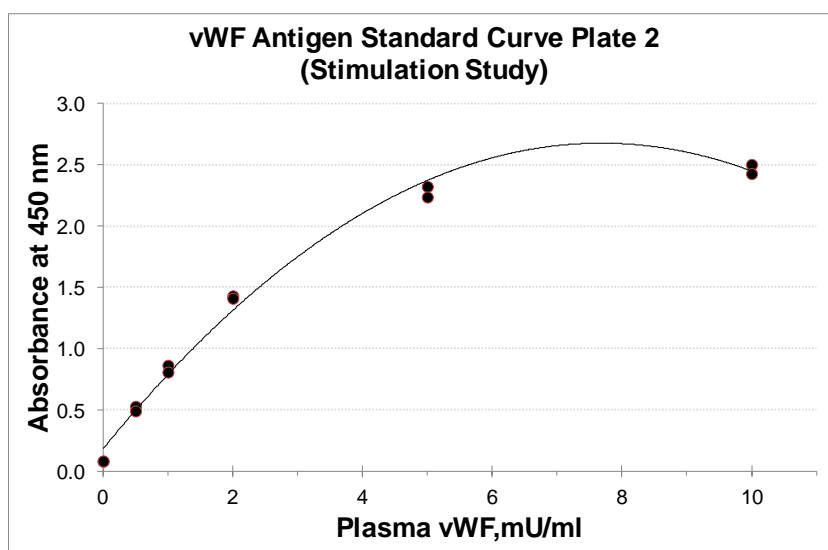
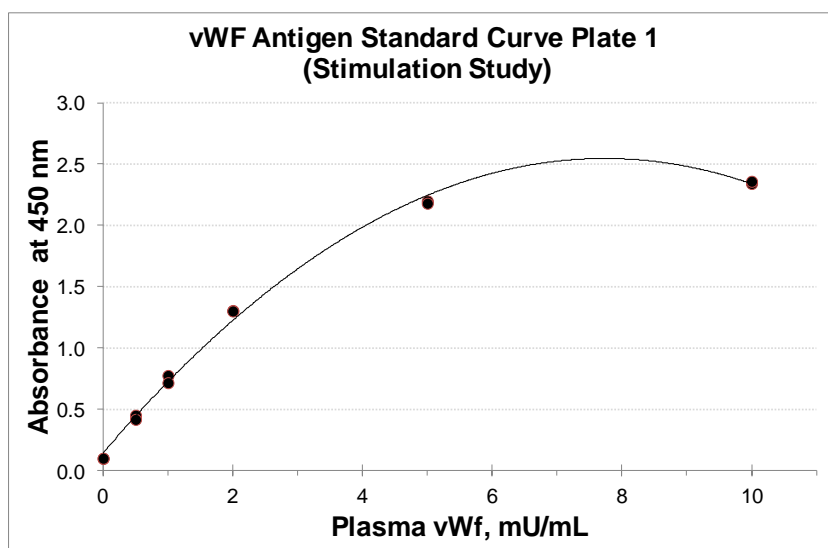


Standard Curves for Plate 3 & 4 (Control Study)

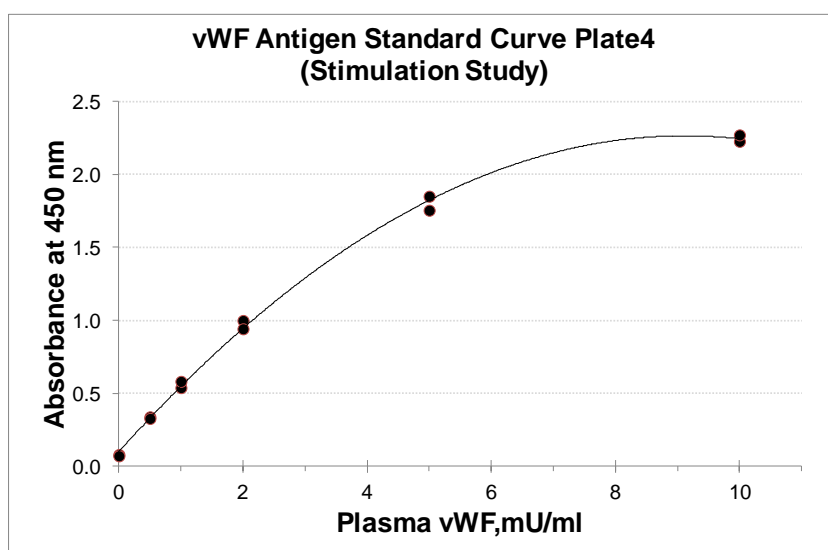
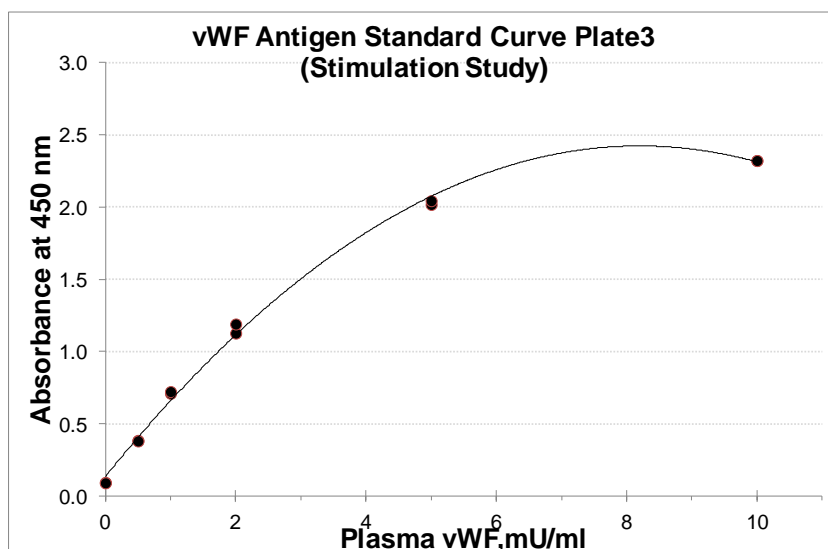


Appendix 18– von Willebrand Factor Antigen Data

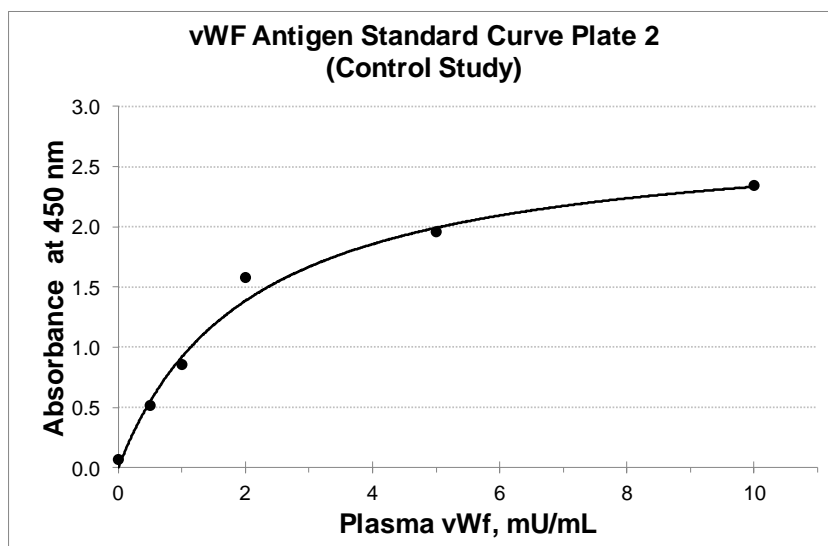
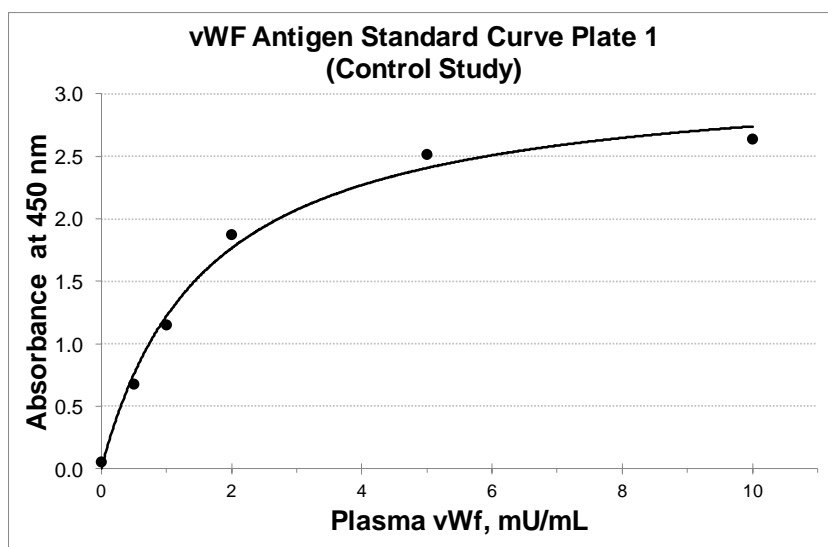
Standard Curves for Plates 1 & 2 (Stimulation Study)



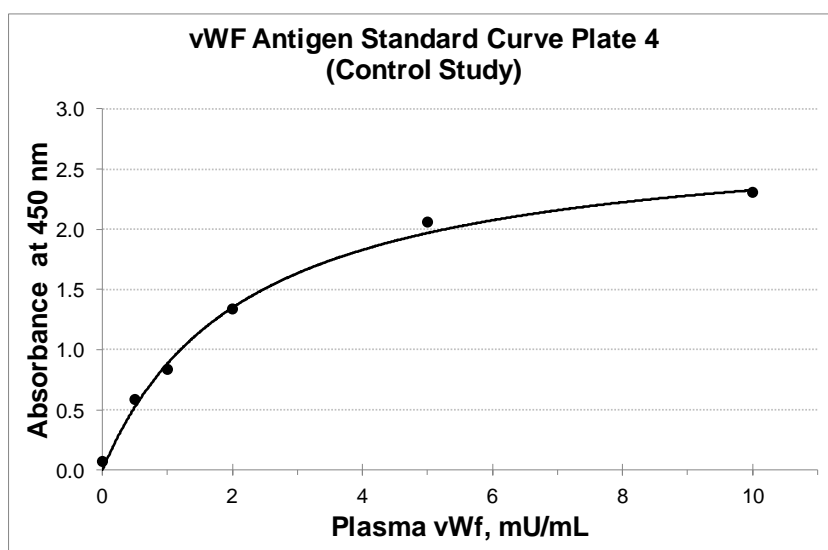
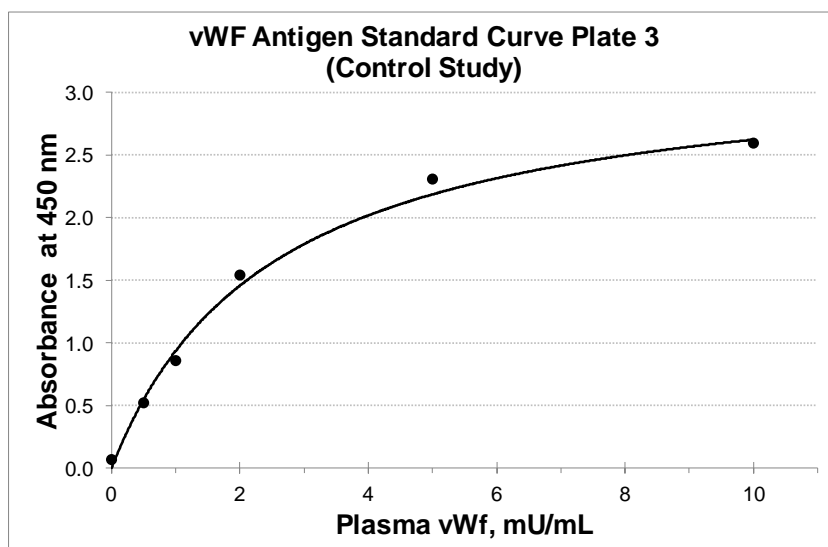
Standard Curves for Plate 3 & 4 (Stimulation Study)



Standard Curves for Plate 1 & 2 (Control Study)

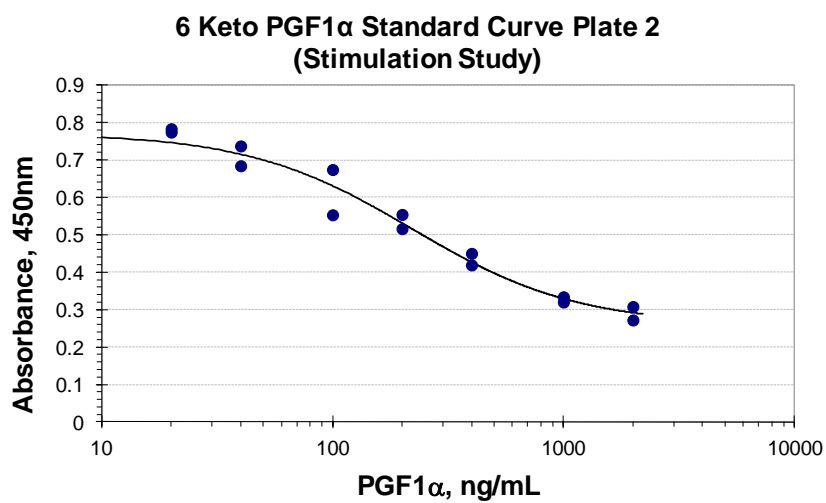
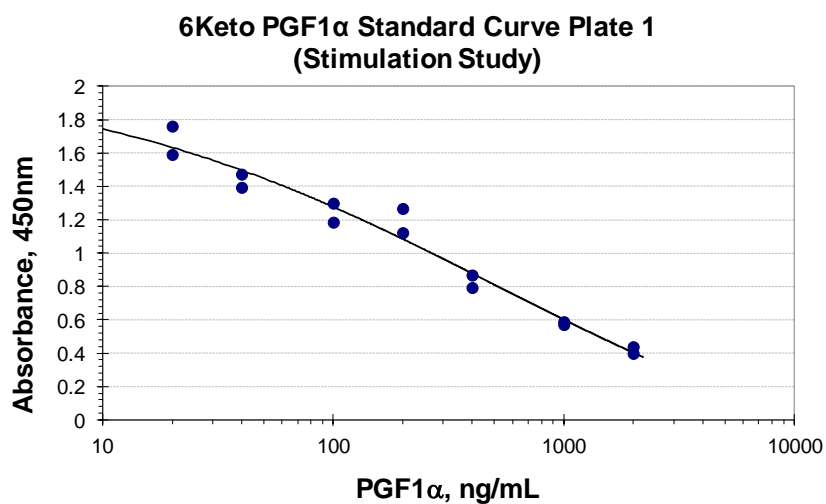


Standard Curves for Plate 3 & 4 (Control Study)

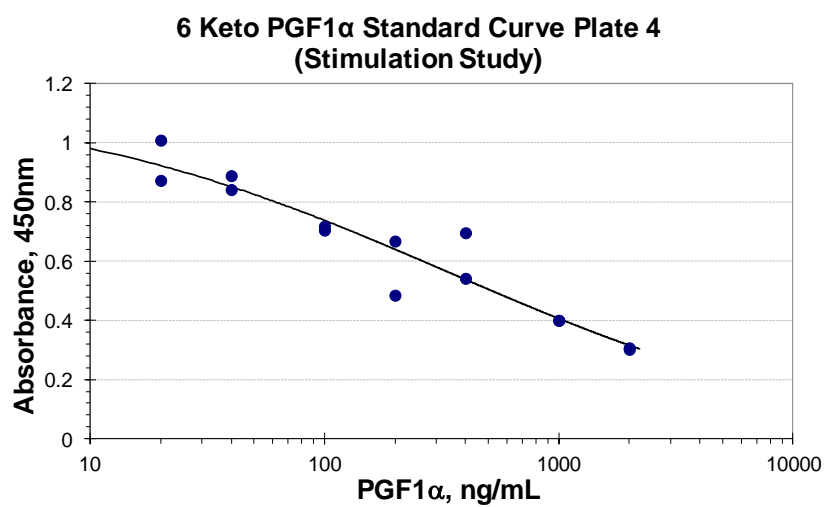
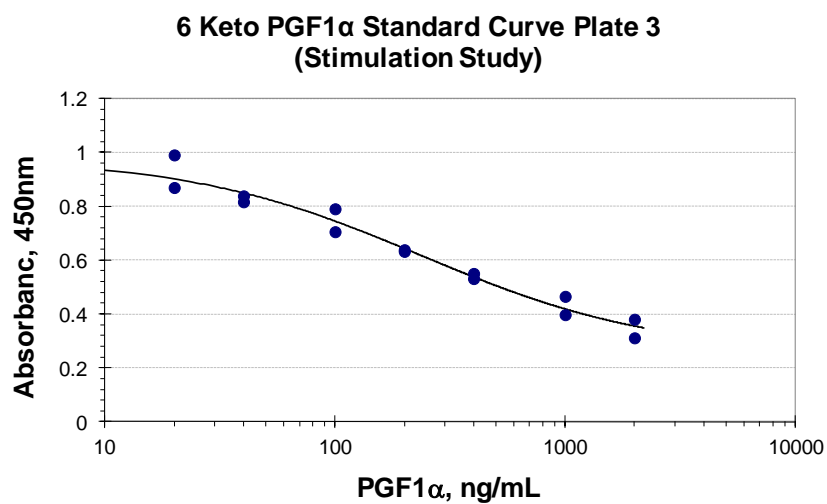


Appendix 19 - 6 Keto PGF1 α Data

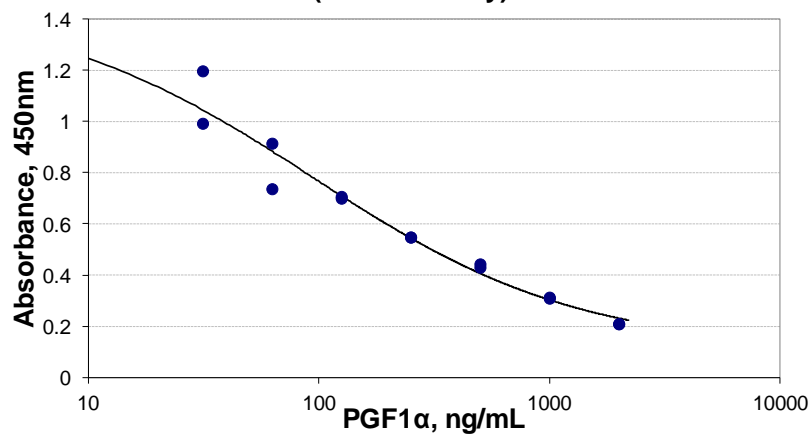
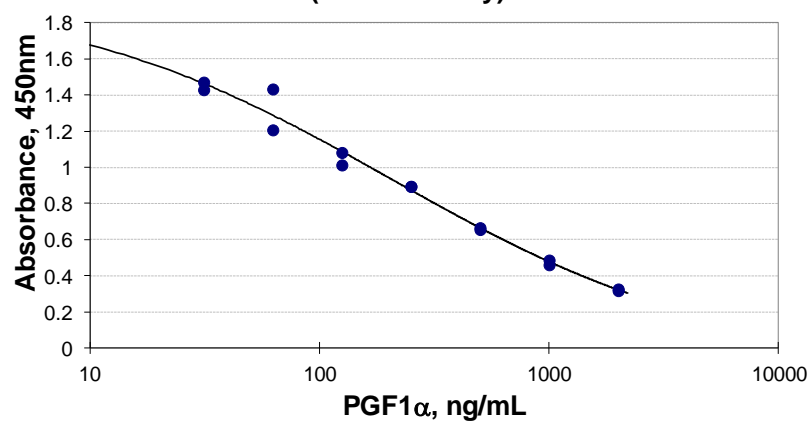
Standard Curve for Plate 1 & 2 (Stimulation Study)



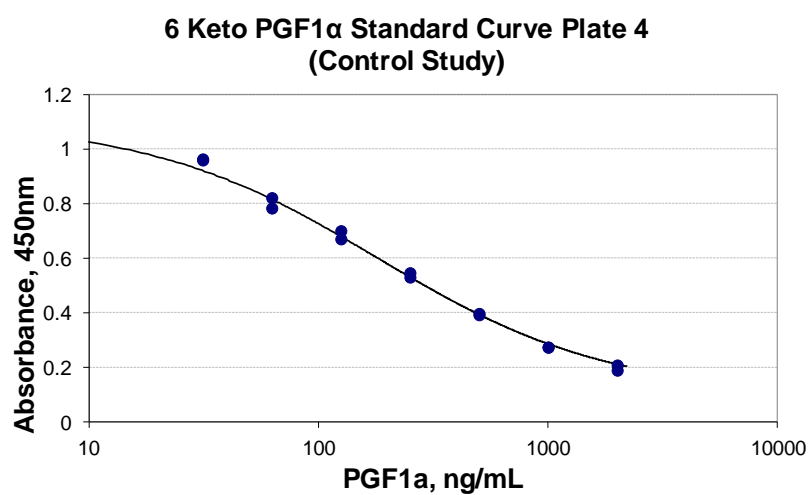
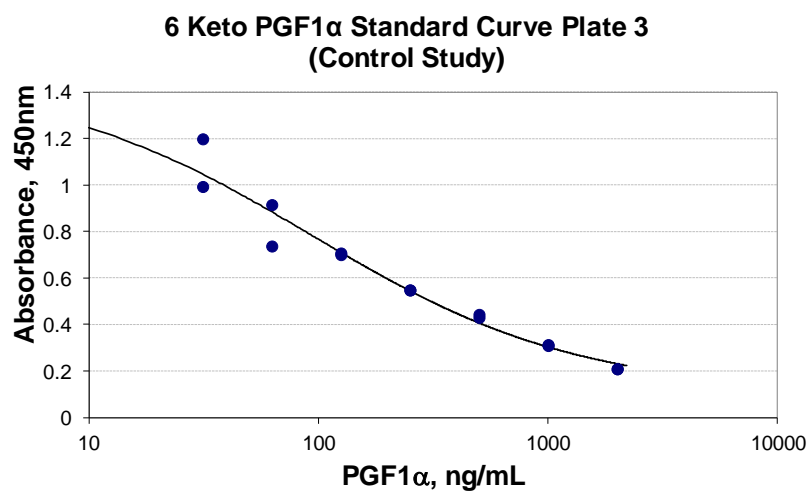
Standard Curve for Plate 3 & 4 (Stimulation Study)



Standard Curve for Plate 1 & 2 (Control Study)

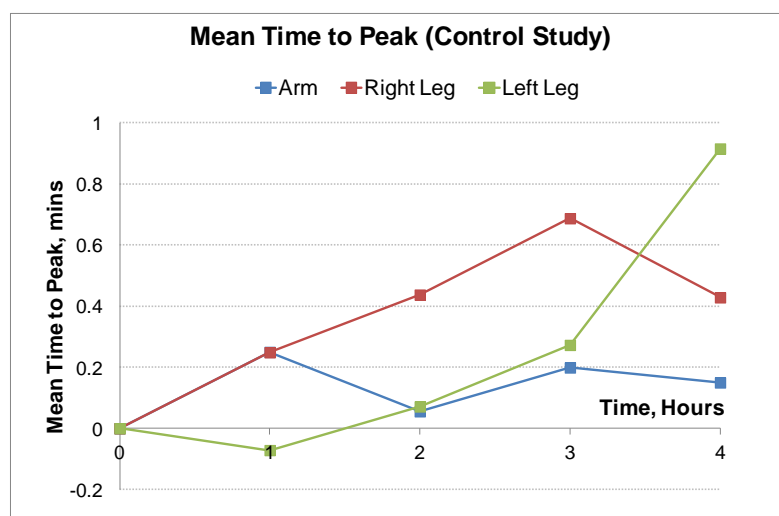
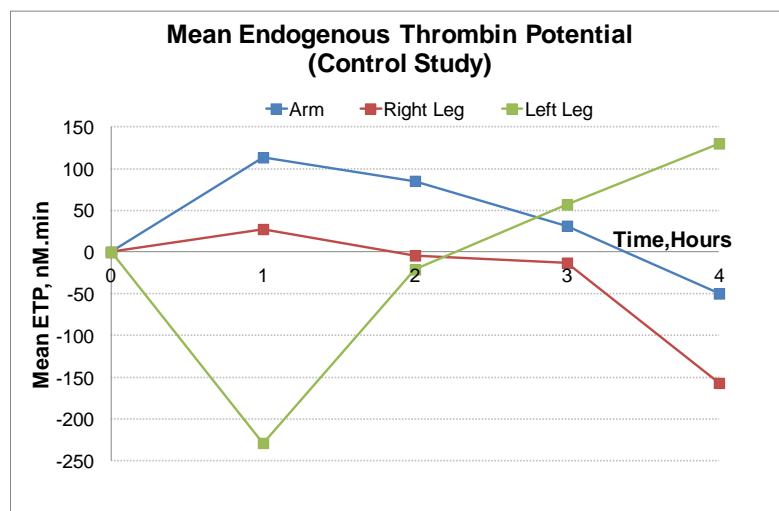
**6 Keto PGF1 α Standard Curve Plate 1
(Control Study)****6 Keto PGF1 α Standard Curve Plate 2
(Control Study)**

Standard Curve for Plate 3 & 4 (Control Study)

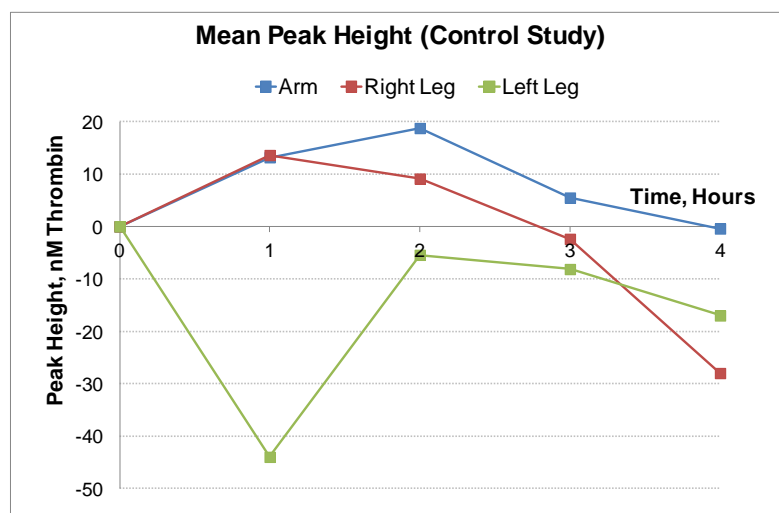


Appendix 20: Thrombin Generation Data

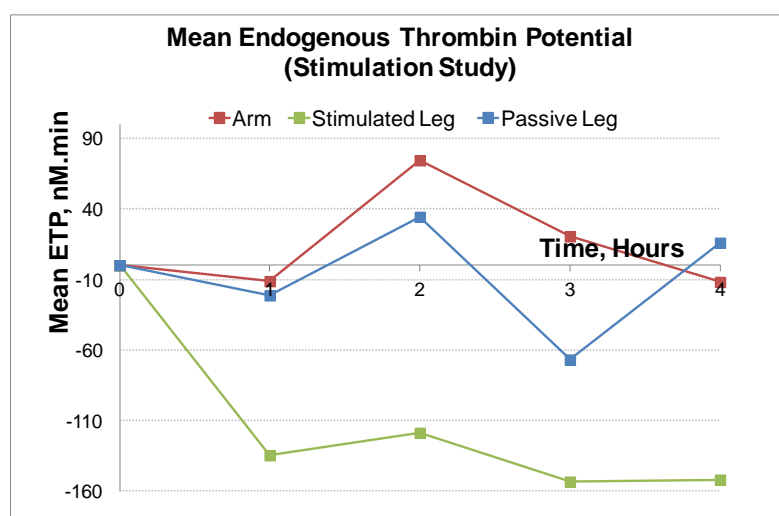
Thrombin Generation – Control Study



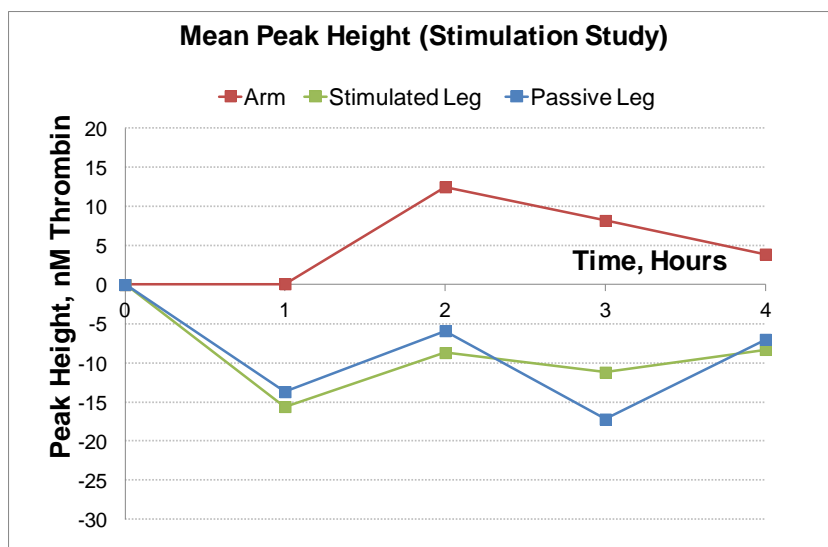
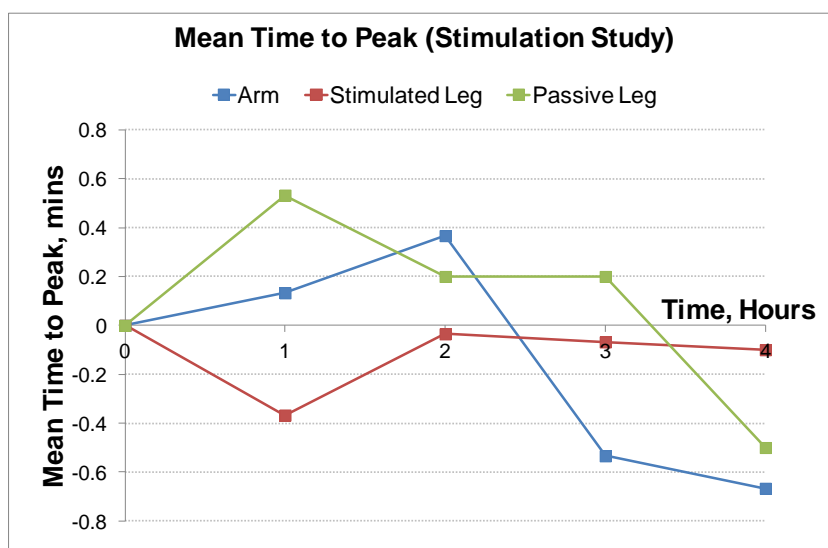
Thrombin Generation – Control Study



Thrombin Generation – Stimulation Study



Thrombin Generation – Stimulation Study



Thrombin Generation & D-dimer Results- Stimulation Study

Subject	Site	Time	D-dlmer	ETP (nM.min)	Peak height (nM thrombin)	Time to peak (mins)	Lag time (mins)
1	Arm	0	<100	3417	552	6.67	3.33
1	Arm	1	<100	3259	539	6.67	3.33
1	Arm	2	<100	4269	649	8.00	4.00
1	Arm	3	<100	3708	593	7.33	3.33
1	Arm	4	<100	2283	370	6.67	3.33
1	Lleg	0	<100	2292	379	7.33	3.33
1	Lleg	1	<100	2374	377	7.33	4.00
1	Lleg	2	<100	2591	417	6.67	3.33
1	Lleg	3	<100	2256	365	7.33	3.33
1	Lleg	4	<100	2494	386	7.33	4.00
1	Rleg	0	<100	2324	375	7.33	4.00
1	Rleg	1	<100	2070	339	6.67	3.33
1	Rleg	2	<100	2253	349	7.33	4.00
1	Rleg	3	<100	2243	350	7.33	4.00
1	Rleg	4	<100	2201	359	7.00	4.00
2	Arm	0	<100	2293	339	8.33	4.67
2	Arm	1	<100	2521	362	9.00	5.00
2	Arm	2	<100	2503	363	9.00	5.00
2	Arm	3	*	*	*	*	*
2	Arm	4	*	*	*	*	*
2	Lleg	0	<100	2398	360	9.00	5.00

Thrombin Generation & D-dimer Results- Stimulation Study

Subject	Site	Time	D-dlmer	ETP (nM.min)	Peak height (nM thrombin)	Time to peak (mins)	Lag time (mins)
2	Lleg	1	<100	2829	388	9.33	5.33
2	Lleg	2	<100	2780	379	9.33	5.33
2	Lleg	3	<100	2516	343	8.67	4.67
2	Lleg	4	<100	2634	372	9.33	5.33
2	Rleg	0	<100	2417	353	9.33	5.33
2	Rleg	1	<100	2329	321	8.67	4.67
2	Rleg	2	<100	2465	342	9.00	5.00
2	Rleg	3	<100	2465	345	9.33	5.33
2	Rleg	4	<100	2280	329	8.33	4.67
3	Arm	0	<100	2627	402	8.67	5.33
3	Arm	1	<100	2637	408	8.67	5.33
3	Arm	2	<100	2560	416	8.67	5.33
3	Arm	3	<100	2629	417	8.67	5.33
3	Arm	4	<100	2883	440	9.33	5.33
3	Lleg	0	<100	2403	411	8.33	5.33
3	Lleg	1	<100	1544	275	6.67	3.33
3	Lleg	2	<100	1667	276	7.33	4.00
3	Lleg	3	<100	1730	285	7.33	4.00
3	Lleg	4	<100	1903	321	7.67	4.00
3	Rleg	0	<100	2452	380	8.67	4.67
3	Rleg	1	<100	2749	419	8.67	5.33

Thrombin Generation & D-dimer Results- Stimulation Study

Subject	Site	Time	D-dlmer	ETP (nM.min)	Peak height (nM thrombin)	Time to peak (mins)	Lag time (mins)
3	Rleg	2	<100	2540	418	8.67	5.33
3	Rleg	3	<100	2742	429	8.67	5.33
3	Rleg	4	<100	2676	423	8.67	5.33
4	Arm	0	<100	1528	264	6.00	3.00
4	Arm	1	<100	1586	278	6.00	3.00
4	Arm	2	<100	1627	286	6.67	3.67
4	Arm	3	<100	1548	296	6.33	3.33
4	Arm	4	<100	2058	367	7.00	3.67
4	Lleg	0	<100	2158	399	7.00	4.00
4	Lleg	1	<100	2041	388	6.67	3.33
4	Lleg	2	<100	2472	444	7.33	4.00
4	Lleg	3	<100	2627	459	8.00	4.67
4	Lleg	4	*	*	*	*	*
4	Rleg	0	<100	2280	392	7.00	4.00
4	Rleg	1	<100	2226	366	7.00	3.67
4	Rleg	2	<100	2127	368	7.00	4.00
4	Rleg	3	<100	2161	376	7.33	4.00
4	Rleg	4	<100	2090	371	7.33	4.00
5	Arm	0	<100	3820	476	9.33	5.33
5	Arm	1	<100	3577	466	9.33	5.33
5	Arm	2	<100	3668	468	9.33	5.33

Thrombin Generation & D-dimer Results- Stimulation Study

Subject	Site	Time	D-dlmer	ETP (nM.min)	Peak height (nM thrombin)	Time to peak (mins)	Lag time (mins)
5	Arm	3	<100	3704	492	9.33	5.33
5	Arm	4	<100	3508	501	8.67	5.00
5	Lleg	0	<100	2391	351	8.00	4.00
5	Lleg	1	<100	2529	358	7.67	3.67
5	Lleg	2	<100	2262	331	7.67	3.67
5	Lleg	3	<100	2037	306	7.00	3.33
5	Lleg	4	<100	2482	356	8.33	4.33
5	Rleg	0	<100	3198	437	8.67	4.67
5	Rleg	1	<100	2110	322	6.67	3.00
5	Rleg	2	<100	2415	349	7.67	4.00
5	Rleg	3	<100	2213	332	7.67	3.67
5	Rleg	4	<100	2267	336	7.33	3.67
6	Arm	0	<100	2577	358	8.00	4.00
6	Arm	1	<100	2254	337	8.00	4.33
6	Arm	2	<100	2317	344	8.00	4.33
6	Arm	3	<100	2314	346	8.00	4.33
6	Arm	4	<100	2309	350	8.00	4.00
6	Lleg	0	<100	2319	348	8.00	4.67
6	Lleg	1	<100	2207	322	8.67	4.67
6	Lleg	2	<100	2184	327	8.67	4.67
6	Lleg	3	<100	2137	327	8.33	4.33

Thrombin Generation & D-dimer Results- Stimulation Study

Subject	Site	Time	D-dlmer	ETP (nM.min)	Peak height (nM thrombin)	Time to peak (mins)	Lag time (mins)
6	Lleg	4	<100	2037	329	7.67	3.67
6	Rleg	0	<100	2230	345	8.00	4.00
6	Rleg	1	<100	2114	326	8.00	4.00
6	Rleg	2	<100	2177	333	8.00	4.33
6	Rleg	3	<100	2282	341	8.67	4.67
6	Rleg	4	<100	2351	342	8.67	4.67
7	Arm	0	<100	1675	298	6.67	3.33
7	Arm	1	<100	1898	318	7.33	3.67
7	Arm	2	<100	1904	325	7.00	3.67
7	Arm	3	<100	1822	317	7.33	4.00
7	Arm	4	<100	1851	321	7.33	4.00
7	Lleg	0	<100	1755	297	6.67	3.33
7	Lleg	1	<100	1769	311	7.33	4.00
7	Lleg	2	<100	1847	314	7.33	4.00
7	Lleg	3	<100	1777	311	7.33	4.00
7	Lleg	4	<100	1792	313	7.33	4.00
7	Rleg	0	<100	1849	308	7.33	4.00
7	Rleg	1	<100	1813	315	7.00	3.67
7	Rleg	2	<100	1743	307	6.67	3.33
7	Rleg	3	<100	1749	318	6.67	3.33
7	Rleg	4	<100	1925	322	7.33	4.00

Thrombin Generation & D-dimer Results- Stimulation Study

Subject	Site	Time	D-dlmer	ETP (nM.min)	Peak height (nM thrombin)	Time to peak (mins)	Lag time (mins)
8	Arm	0	<100	3118	477	6.00	3.33
8	Arm	1	<100	3249	483	6.00	3.33
8	Arm	2	<100	3102	439	6.67	3.33
8	Arm	3	<100	3102	450	6.67	3.33
8	Arm	4	<100	3272	466	6.67	3.33
8	Lleg	0	<100	3492	543	6.33	3.33
8	Lleg	1	<100	3358	516	6.33	3.33
8	Lleg	2	<100	3431	516	6.67	3.33
8	Lleg	3	<100	3257	501	6.67	3.33
8	Lleg	4	<100	3442	512	6.33	3.33
8	Rleg	0	<100	3122	456	6.67	3.33
8	Rleg	1	<100	3079	453	6.67	3.33
8	Rleg	2	<100	2917	458	6.33	3.33
8	Rleg	3	<100	2779	457	6.00	3.33
8	Rleg	4	<100	2997	477	6.00	3.00
9	Arm	0	<100	2492	354	8.33	4.33
9	Arm	1	<100	2531	363	8.00	4.00
9	Arm	2	<100	2491	357	8.33	4.00
9	Arm	3	<100	2780	372	8.67	4.67
9	Arm	4	<100	2868	386	8.00	4.00
9	Lleg	0	<100	2473	336	8.00	4.00

Thrombin Generation & D-dimer Results- Stimulation Study

Subject	Site	Time	D-dimer	ETP (nM.min)	Peak height (nM thrombin)	Time to peak (mins)	Lag time (mins)
9	Lleg	4	<100	2279	305	8.67	4.00
9	Rleg	0	<100	2724	347	9.33	4.67
9	Rleg	1	<100	2833	395	8.33	4.33
9	Rleg	2	<100	2715	376	8.67	4.67
9	Rleg	3	<100	2502	344	8.67	4.00
9	Rleg	4	<100	2387	343	8.00	4.00
10	Arm	0	148	2258	411	6.67	4.00
10	Arm	1	402	2180	378	7.00	4.00
10	Arm	2	<100	2108	398	6.67	4.00
10	Arm	3	<100	1900	367	7.00	4.00
10	Arm	4	<100	2151	406	7.00	4.33
10	Lleg	0	118	2105	409	6.67	4.00
10	Lleg	1	<100	2241	406	7.33	4.00
10	Lleg	2	<100	2390	446	7.33	4.67
10	Lleg	3	<100	2306	400	8.00	4.67
10	Lleg	4	<100	2255	410	7.67	4.67
10	Rleg	0	140	2265	405	6.67	4.00
10	Rleg	1	<100	2190	386	6.67	3.67
10	Rleg	2	<100	2320	411	7.33	4.33
10	Rleg	3	<100	2190	394	7.33	4.33
10	Rleg	4	<100	2166	413	7.33	4.33

Appendix 21 – Ethics Approval Letter for geko™ versus IPC Comparison Study


National Research Ethics Service
North London REC 1

 Room 019, Level 7 Maternity Block
 Northwick Park Hospital
 Watford Road
 Harrow
 Middlesex
 HA1 3UJ

Tel: 020 8869 3805

Fax: 020 8869 5222

07 January 2011

 Dr Arthur Tucker
 Clinical Scientist & Senior Lecturer
 The Ernest Cooke Clinical Microvascular Unit
 4th Floor Dominion House
 St. Bartholomew's Hospital
 London
 EC1A 7BE

Dear Dr Tucker

Study title:	A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Subjects
REC reference:	05/Q0408/14
Protocol number:	Thrive-1
EudraCT number:	Not applicable
Amendment number:	Sub AM05 – Amendment 7.0, Dated 28-12-2010
Amendment date:	28 December 2010

Thank you for submitting the above amendment, which was received on 05 January 2011. I can confirm that this is a valid notice of a substantial amendment and will be reviewed by the Sub-Committee of the REC at its next meeting.

Documents received

The documents to be reviewed are as follows:

Document	Version	Date
European Commission Notification of Substantial Amendment Form		28 December 2010
Covering Letter		28 December 2010
Participant Information Sheet: IPC Sub-Group	2.0 - Tracked	28 December 2010
Participant Information Sheet: IPC Sub-Group	2.0 - Clean	28 December 2010
Participant Consent Form: IPC Sub-Group	2.0	28 December 2010
Protocol Amendment 7 Summary	1.8.2	28 December 2010
Protocol	1.8.2	28 December 2010

Notification of the Committee's decision

The Committee will issue an ethical opinion on the amendment within a maximum of 35 days from the date of receipt.

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
 The National Research Ethics Service (NRES) represents the NRES Directorate within
 the National Patient Safety Agency and Research Ethics Committees in England

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval for the research.

05/Q0408/14:	Please quote this number on all correspondence
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Yours sincerely



Mr Lawrence L. Penez
Committee Co-ordinator

E-mail: lawrence.penez@nwlh.nhs.uk

Copy to:

Mr Gerry Leonard, Barts and The London NHS Trust, Joint-Research Office,
3rd Floor, Rutland House, 42-46 New Road, The Royal London, Hospital,
Whitechapel, London E1 1BE

Appendix 22 – Subject Information Sheet for geko™ vs. IPC Comparison Study**A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Volunteers**

Lay Title: “THRIVE-1 – IPC Sub-Group”

Part 1**Invitation**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Queen Mary, University of London (QMUL) is the sponsor of this research.

What is the purpose of the study?

You are being asked to take part in this study because you are an adult, aged between 18 and 65 years.

Deep vein thrombosis (DVT) is the formation of a thrombus (clot) within the deep veins. It is a relatively common condition in patients receiving medical or surgical treatment in hospital. Recently there has been much controversy and media interest regarding the development of deep vein thrombosis and its effects in passengers undertaking long haul air travel. This is also referred to as the ‘economy class syndrome’ or ‘travellers thrombosis’ as it has also been associated with other modes of travel e.g. coach and car travel

The THRIVE-I study is examining a new device and method for increasing blood flow in the legs using very low level electrical stimulation. The device is called GEKO™ and is made by Sky Medical Technology Ltd. We know that increases in leg blood flow improve the return of blood towards the heart and thereby reducing accumulation of blood in the legs (venous stasis). Since stasis is one of the important factors in the

development of deep vein thrombosis, the risk of developing thrombosis should be reduced. This technique may prove to be a useful tool in preventing and reducing the incidence of deep vein thrombosis in both medical and surgical patients.

Following completion of the first phase of 50 healthy Volunteers, we have found that the device can significantly increase leg blood flow in the arteries, veins and small blood vessels of the legs. The increases appear to be clinically valuable in relation to rest, but within normal ranges compared to exercise. Furthermore, there were no adverse events or causes for concern.

One of the existing methods used in hospitals for the prevention of deep vein thrombosis is intermittent pneumatic compression (IPC) which consists of calf length garments which are pneumatically inflated by a pump for approximately twelve seconds every minute. By gently inflating cuffs placed around the calves, IPC mimics the process of walking by squeezing the calf muscle and forces blood back to the heart. This active compression on the limb stops blood from pooling in the lower limbs and aims to generate continual movement of blood through the veins. It has been found that IPC devices are effective in reducing the risk of DVT by 60% when compared to patients receiving no medical device or drug.

This sub-study will allow us to compare the effect of the geko[®] device with IPC devices to study the increases in blood flow in the legs in 10 healthy Volunteers and 10 Patient Volunteers with Venous Insufficiency. Chronic venous insufficiency is impaired venous return, sometimes causing lower extremity discomfort, swelling, and skin changes. The value of this additional study will be to further confirm the safety and potential of the devices for future studies.

Why have I been chosen?

You are being asked to take part in this study because you are a healthy adult, aged between 18 and 65 year; with or without Venous Insufficiency of your legs.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. As a patient, non-participation or dropping out of the study will not affect your clinical management in any way. If you are employed by Barts & The London NHS Trust or Barts & The London School of Medicine & Dentistry, non-participation or dropping out of the study will not affect your training or career.

Before you can begin the study

You may read the full study protocol as well as this Information sheet, which gives you many details about the study. The recruiting Investigator will tell you about any potential adverse events that could occur in this study. You will be told exactly what the study entails and what will be required of you. You are encouraged to ask

questions of the Investigators conducting the recruitment interview until you are satisfied that you fully understand the nature of the study and the requirements.

What will happen to me if I take part?

Screening

If you agree to take part and sign a **Consent for Study Participation** you will be invited to a selection session. We will take a detailed medical history and a physical examination, including blood pressure, pulse rate and heart rhythm. Signs of drug and alcohol abuse will also be checked. We will use an ultrasound machine to look at the blood vessels in your legs to check for any abnormalities, which might prevent you joining the study. This is entirely painless, and you will be required to attend with a pair of shorts.

Study

If all results match our inclusion and exclusion criteria you will be invited to join the study and you will be asked to start within 7 days. The duration of your participation in the study will be around 3½ hours in total.

We hope to recruit 20 people to this part of the study. On the day of the study you will need to attend the Microvascular Unit at St. Bartholomew's Hospital with a pair of shorts to wear during the testing. You will be instructed to have a light breakfast, avoiding fatty foods, tobacco and caffeine and will be asked to abstain from vigorous exercise from the previous evening onwards. You will be asked to rest for 30 minutes to relax and get used to the environment. Your resting blood flow will then be measured in your legs. These measurements are entirely non-invasive and painless.

We will apply the IPC cuffs around your calves and run the inflation device for 30 minutes and then repeat the measurements. You will have a 'rest period' of ten minutes of rest before the next device is tested after 30 minutes. We will study two routine IPC devices (Huntleigh Flowtron® and the Kendle ICD Express®). After a further ten minutes of rest, we will remove the cuffs from your legs and apply the GEKO® device to the back of each of your knees over a nerve. When switched on, the stimulation will make the muscles of your calf and foot gently contract. This will not hurt, but might feel a little strange and slightly uncomfortable. The device will be turned on for the next 30 minutes, during which time the blood flow measurements will be repeated with a ten minute rest period before repeating. We will test two different settings of the GEKO® device. Also, during these times, we will ask you a series of questions about how each device feels and how comfortable you are compared to the discomfort you associate with having your blood pressure measured.

After this point you will be discharged from the Study.

Expenses and payments

For attending the Screening session we will compensate you with £15-00. At the end of the study we will be pleased to pay you £85-00 for your time, commitment to the study and expenses (a total maximum payment of £100-00).

If the study is stopped by the Investigator(s) prior to completion, if you withdraw or are withdrawn from the study before completion, a pro-rata payment will be made at the discretion of the Investigator(s).

What are the possible benefits of taking part?

This study will not benefit you directly, however it may lead to the development of a novel method for preventing deep vein thrombosis in patients and travellers alike; and may help in the future management of patients to prevent DVT.

Could I come to any harm if I take part in the study?

You may be withdrawn from the study if the doctors feel it is best for you or if you do not comply with the requirements of the study.

If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel unacceptable discomfort, or for any reason during the study you do not wish to continue then we will stop the tests immediately.

All of the previous work using these devices was found to be safe. When the geko[®] device is applying an electrical stimulation, you may feel some muscle twitching and maybe tingling in your lower legs. There are very few risks involved in using this type of equipment and the device is commonly used for therapeutic purposes to exercise muscles under the supervision of a Physiotherapist, as well as by members of the public for “toning” purposes in their own homes.

When the IPC devices are inflating, you will feel the cuffs gently squeezing your calves.

The blood flow measurements and ultrasound are non-invasive, painless and known to be entirely safe.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

If you have a complaint please contact the following in the first instance: Dr. Arthur Tucker.

If you feel any discomfort or distress during the investigations, you must say so and we will stop the tests immediately at any time.

If you are employed by Barts & The London NHS Trust or Barts & The London School of Medicine & Dentistry, non-participation or dropping out of the study will not affect your training or career.

As a patient, non-participation or dropping out of the study will not affect your clinical management in any way

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

A contact number for complaints will be given.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact Details:

If you require any further information please contact:

Dr Arthur Tucker

The Ernest Cooke Vascular & Microvascular Unit,
4th Floor Dominion House,
St. Bartholomew's Hospital,
West Smithfield.
London. EC1A 7BE.
Tel Number: 0203 4656773
This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will need to use the data collected up to your withdrawal.

What if there is a problem?

Queen Mary University of London has agreed that if you are harmed as a result of your participation in the study, you will be compensated, provided that, on the balance of probabilities, an injury was caused as a direct result of the intervention or procedures you received during the course of the study. These special compensation arrangements apply where an injury is caused to you that would not have occurred if you were not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

Complaints

If you have a concern about any aspect of this study, you should ask to speak with the Research Team who will do their best to answer your questions (Dr Arthur Tucker, telephone 0203 4656773).

If you remain unhappy and wish to complain formally you can do this by contacting: The Complaints Officer, c/o The Chief Operating Officer for the Barts and The London, Queen Mary School of Medicine and Dentistry, Wardens Office, 32 Newark Street, Whitechapel, London E1 2AA, telephone 020 7882 2259.

Will my taking part in this study be kept confidential?

All the information obtained about you in the course of the study is confidential and will be kept in a secure locked room. The investigators performing the study and a study Monitor will have access to the data collected in this study. They may also be looked at by representatives of regulatory authorities and by authorised people from Queen Mary University of London Medical School, Barts & The London NHS Trust, Sky Medical Technology or the Regulatory Authorities to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen to the results of the research study?

The results of this study may be published or presented at meetings. You will not be identified in any report / publication or presentation.

Who is organising and funding the research?

This research is being sponsored by Queen Mary, University of London Medical School.

Who has reviewed the study?

This study has been given a favourable ethical opinion for conduct in the NHS by North London REC 1 (formerly known as Brent) Research Ethics Committee reference:05 / Q0408 / 14.

Before you sign this consent form please ask any questions you have about the study.

Thank you for taking the time to read this information sheet.

Appendix 23 – Participation Consent Form for geko™ vs IPC Comparison Study

WRITTEN CONSENT FORM:

REC Number: 05 / Q0408 / 14

ver 2.0

Title of research proposal: A study to determine the effects of a novel method for improving lower limb blood flow in Healthy Adult Volunteers –

IPC Comparison Sub-Group
(Protocol number: THRIVE-1)

Name of Researcher: Dr Arthur T. Tucker

Name of Patient / Volunteer (Block Capitals):

Address:

STUDY CONSENT FORM

(Initial boxes to agree with statement)

1. I confirm that I have read and understand the THRIVE-1 – IPC Comparison Sub-Group information sheet **dated 28th December 2010 version 2.0**, for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐

1. I understand that my participation of the **STUDY** is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐

2. I understand that relevant sections of any of my data generated during the study may be looked at by responsible individuals from Queen Mary University of London Medical School, Barts & The London NHS Trust, Sky Medical Technologies and from the regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

☐

3. I agree to my GP being informed of any significant information relevant to my participation.

☐

4. I agree to take part in the above study.

☐

 Name of Patient

 Date

 Signature

 Name of Person taking consent
 (if different from researcher)

 Date

 Signature

 Researcher

 Date

 Signature

When completed, 1 for Research Subject and 1 for researcher site file (original)

Appendix 24 – Randomisation Sequence for Device Fitting

<i>Subject</i>	<i>Device Sequence</i>
1	IPC 1, IPC 2, geko™ 1, geko™ 2
2	geko™ 1, geko™ 2, IPC1, IPC 2
3	IPC 1, IPC 2, geko™ 1, geko™ 2
4	geko™ 1, geko™ 2, IPC1, IPC 2
5	IPC 1, IPC 2, geko™ 1, geko™ 2
6	geko™ 1, geko™ 2, IPC1, IPC 2
7	IPC 1, IPC 2, geko™ 1, geko™ 2
8	geko™ 1, geko™ 2, IPC1, IPC 2
9	IPC 1, IPC 2, geko™ 1, geko™ 2
10	geko™ 1, geko™ 2, IPC1, IPC 2
11	IPC 1, IPC 2, geko™ 1, geko™ 2
12	geko™ 1, geko™ 2, IPC1, IPC 2
13	IPC 1, IPC 2, geko™ 1, geko™ 2
14	geko™ 1, geko™ 2, IPC1, IPC 2
15	IPC 1, IPC 2, geko™ 1, geko™ 2
16	geko™ 1, geko™ 2, IPC1, IPC 2
17	IPC 1, IPC 2, geko™ 1, geko™ 2
18	geko™ 1, geko™ 2, IPC1, IPC 2
19	IPC 1, IPC 2, geko™ 1, geko™ 2
20	geko™ 1, geko™ 2, IPC1, IPC 2
21	IPC 1, IPC 2, geko™ 1, geko™ 2
22	geko™ 1, geko™ 2, IPC1, IPC 2
23	IPC 1, IPC 2, geko™ 1, geko™ 2
24	geko™ 1, geko™ 2, IPC1, IPC 2
25	IPC 1, IPC 2, geko™ 1, geko™ 2

Device Code

IPC Huntleigh = IPC 1

IPC Kendle = IPC 2

geko™ Lower Setting = geko™ 1

geko™ Higher Setting 2 = geko™ 2

Appendix 25 – geko™ T-1 device Manual

T-1

G-IL-T1-v1 / 28-06-2010

geko™
circulation support

instructions for use – daily disposable – replace every 24 hours

The geko™ device may be fitted to either or both legs as prescribed by the physician.

The device should be fitted so that the control button is secured to the outside of the leg.

centre crease

outer tendon

1 First find the location behind and to the outside of the knee where the device is to be secured, identifying the **outer tendon** and the **centre crease** at the back of the knee.

2 Using the grey abrasive pad provided and, without breaking the skin, apply light pressure to **exfoliate the area** where the device is to be secured – then **wipe the area thoroughly** with the electrode preparation wipe.

position just above centre crease

align indicator with tendon

3 After 30 seconds, peel off the protective film and **secure the device to a straight leg** so that:

- the lower edge of the device is positioned very **slightly above the crease of the knee**
- the raised **indicator line** on the device marked with arrows is **aligned with the outer tendon**
- the **control button** is secured on the **outside of the knee**

4 Use a **short press (click) of the button** to activate the device and subsequently set the appropriate level (indicated by the flashing light) – as detailed in the accompanying instructions – to cause minor visible movement of the calf and foot. There are a total of 7 levels. In most cases levels 3 or 4 are ideal.

▲ **basic fitting guide** – to be viewed in conjunction with the full instructions included in this leaflet

geko™ devices are used to increase venous circulation and for the prevention of venous thrombosis

geko™ devices should only be worn at the knee

OnPulse™
TECHNOLOGY

geko™ instructions for use

1.0 geko™ device

1.1 Its purpose

The geko™ device is a neuromuscular stimulation device and its intended use is:

- to increase venous circulation, and
- for the prevention of venous thrombosis

1.2 Description of geko™ device

The geko™ is a disposable, internally powered, neuromuscular stimulation device. It is an integrated device and there are no accessories required for its operation. Familiarise yourself with the components before you use the device and the skin preparation materials.

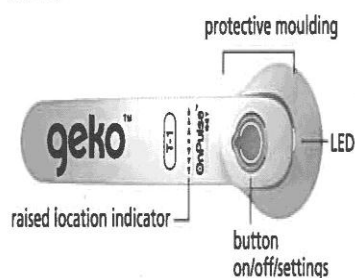


fig 1.1 topside of geko™

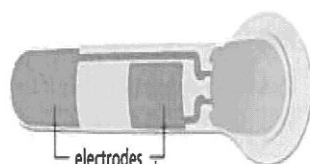


fig 1.2 underside of geko™

table 1.1 Items that make up the geko™

component	description
protective moulding	A covering to house its internal electronics and battery and protect the device from damage when stored or in use.
button	Pushing the button switches

table continued...

not flash. If a fault occurs, such as current levels outside normal operating parameters or the battery voltage is low, the unit will switch off. The unit will also switch off when it has run for its maximum working time of 30 hours.

disposable protective backing film	Peel off backing film that protects the hydrogel pad from drying out.
hydrogel	Water based conductive gel that makes electrical connection between the electrode and the patient's skin.
electrodes	Printed electrodes covered with conductive hydrogel to allow efficient passing of the electrical stimulus to the nerve(s).

table 1.2  Response of pushing the button on the geko™ device

action	response
short push (click) on button	Device switches on. Low stimulation level set. Indicator light flashes once then repeats after 1.5 seconds.
subsequent short push (click) on button	The level of stimulation is increased to the next level. The LED flashes to indicate level (1 to 7), then repeats after a gap of 1.5 seconds.
medium length push on button (click and hold for 1 to 2 seconds)	The stimulation intensity decreases by one level. The number of LED flashes indicate the level.
long push on	Device switches off.

1.3.1 Warnings:

- The long-term effects of chronic electrical stimulation are unknown.
- Stimulation should not be applied over the carotid sinus nerves, particularly in patients with a known sensitivity to the carotid sinus reflex.
- Stimulation should not be applied over the neck or mouth. Severe spasm of the laryngeal and pharyngeal muscles may occur and the contractions may be strong enough to close the airway or cause difficulty in breathing.
- Stimulation should not be applied transthoracically in that the introduction of electrical current into the heart may cause cardiac arrhythmias.
- Stimulation should not be applied transcranially.
- Stimulation should not be applied over swollen, infected, or inflamed areas or skin eruptions, e.g. phlebitis, thrombophlebitis, varicose veins etc.
- Stimulation should not be applied over, or in proximity to, cancerous lesions.
- The geko™ device is self-adhesive and, in a few cases, skin inflammation or irritation can develop in the contact area. Switch off the device and remove.
- geko™ devices should not be used for more than 28 days continuously without specific instructions from the consulting physician.
- Simultaneous connection of a patient to high frequency surgical equipment may result in burns at the site of stimulator electrodes and possible damage to the stimulator.
- Operation in close proximity (e.g. one metre) to a shortwave or microwave equipment may produce instability in the stimulator output.
- The geko™ device has no replaceable or serviceable parts and requires no maintenance or calibrations. The unit must not be disassembled.
- No modification of this device is allowed.

- The geko™ device must be kept dry. The patient must not bath or shower while wearing a geko™ device.
- Damaged geko™ devices or devices with damaged packaging must not be used.

1.3.2 Precautions

- Safety of powered muscle stimulators for use during pregnancy has not been established.
- Caution should be used for patients with suspected or diagnosed heart problems.
- Caution should be used for patients with suspected or diagnosed epilepsy.
- Caution should be used in the presence of the following:
 - a) when there is a tendency to haemorrhage following acute trauma or fracture;
 - b) following recent surgical procedures when muscle contraction may disrupt the healing process;
 - c) over the menstruating or pregnant uterus; and
 - d) over areas of the skin which lack normal sensation.
- Some patients may experience skin irritation or hypersensitivity due to the electrical stimulation or electrical conductive medium. The irritation can usually be reduced by using an alternate electrode placement; try lowering the device so the upper edge is positioned very slightly below the crease of the knee.
- Electrode placement and stimulation settings should be based on the guidance of the prescribing practitioner.
- Powered muscle stimulators should be kept out of the reach of children.
- Powered muscle stimulators should be used only with the leads and electrodes recommended for use by the manufacturer.
- Portable powered muscle stimulators should not be used while driving, operating machinery, or during any activity in which involuntary muscle contractions may put the user at undue risk of injury.

1.3.3 Adverse reactions

Skin irritation and burns beneath the electrodes have been reported with the use of powered muscle stimulators.

1.3.3 Reporting of any side effects or adverse reactions

If the geko™ device is being used for its intended

purpose and according to the instructions, any side effects or adverse reactions should be reported to Firstkind Limited. The use of the stimulator for that patient should be suspended until further investigations have been carried out.

2.0 About the geko™ device and muscle stimulation

2.1 The physiology

The circulatory system serves to transport and distribute essential substances to the tissues of the body and to remove by-products of metabolism. It also plays a role in the regulation of body temperature, humoral communication throughout the body and adjustments of oxygen and nutrient supply in differing physiological states. The cardiovascular system is made up of a pump (the heart), a series of distributing and collecting tubes and an extensive system of thin vessels that allow rapid exchange with tissues. An average adult has a blood volume of about 5-6 litres. The venous system has a large capacity and may contain some 70% of the blood volume at any time with a large percentage of this in the lower legs. Cardiac output is the volume of blood pumped by the heart per minute and venous return is the volume returning to the heart in the same unit of time. These are interdependent and multiple feedback control loops operate to regulate the cardiovascular system. Ancillary factors can affect venous return including muscular activity. Contraction of the muscles causes intermittent venous compression and, because of the orientation of the venous valves, blood is forced from the veins toward the heart. Therefore, muscular contraction lowers the mean venous pressure and serves as an auxiliary pump to assist venous return. Muscle contraction lowers capillary hydrostatic pressure and increases local blood circulation and prevents venous pooling (or stasis).

2.2 How geko™ works

The geko™ device is a small disposable, internally powered, neuromuscular stimulation device that is applied externally to the leg. It is self-adhesive and is applied to the outer/posterior aspect of the knee. This positioning enables integral electrodes to apply a stimulus to the lateral and medial popliteal nerves which branch from the...

continued overleaf

T-1 instructions for use

continued from overleaf

... sciatic nerves. These nerves control the contraction of the foot, shin and calf muscles. The stimulation of these nerves by the geko™ device causes the muscles to isometrically contract and will not affect normal movement of the limb nor mobility of the patient. Contraction of the lower leg muscles will boost blood flow from the lower limbs back to the heart thus increasing venous return, local blood circulation and help prevent venous thrombosis. The geko™ device has seven stimulation levels to balance maximal effect of stimulation with patient comfort. It is fully insulated by the protective moulding and there is no risk of shock.

2.3 The patient experience

The application of the geko™ device is very simple and the patient will only experience a cooling effect as the area of skin, to which the device will be applied, is cleaned. Thereafter, the patient will feel as if a small adhesive patch has been applied to the skin.

Upon switching on the geko™ device and selecting the appropriate stimulation level, the patient will be aware of the muscle contraction, awareness of which will recede slightly after a few minutes (accommodation).

Over the next hour and the 24 hour treatment period the patient's awareness of muscle contraction will lessen and the patient can carry out their normal routine including sleep.

3.0 Operating information for healthcare professionals

Carefully read all instructions before applying and using the device. If the geko™ device has been stored at low temperatures (below 5°C), allow to reach room temperature before use.

3.1 Preparation

▼ 1 Check any contraindications, warnings and precautions for use of the device with the particular patient

▼ 2 Position the patient so that they are comfortable, their lower leg is supported and you can access the outer/posterior aspect of the knee. The knee should be straight not bent.

▼ 3 Find the location behind and to the outside of the knee where the device is to be secured, identifying the outer tendon and the centre crease at the back of the knee. You need to be able to

clearly see the indicator light of the device which will be at the outer aspect of the leg when applied.

▼ 4 Using the grey abrasive pad provided and, without breaking the skin, apply light pressure to exfoliate the area where the device is to be secured. Follow this by wiping the area thoroughly with the electrode preparation wipe and leave for 30 seconds.

If this application of the geko™ device is to immediately replace a geko™ device, just wipe the area thoroughly with the electrode preparation wipe. The grey abrasive pad should not be needed.

3.2 Application

▼ 5 Peel off the protective film and secure the device to a straight leg so that:

- the tongue of the device is on the back of the knee and the lower edge of the device is positioned very slightly above the crease of the knee
- the raised indicator line on the device marked with arrows is aligned with the outer tendon
- the control button is secured on the outside of the knee

3.3 Operating instructions

▼ 6 Turn on the device by pressing the button once (click). The LED will flash regularly every 1.5 seconds to signal that it is operating correctly and that the low level of stimulation is set.

▼ 7 To advance the setting to a higher level, with a short click, press the button on the device again. The LED will flash in quick succession to signal the level of the stimulation that has been set. There are seven levels.

▼ 8 Adjust the setting upwards in this way until the foot twitches outwards. The level is correct when the foot moves slightly when raised from the ground. Levels 3 and 4 of the device are the most likely setting to achieve this effect.

▼ 9 If the stimulation is too high, press the button for a time between one and two seconds (click and hold) and this will reduce the stimulation by one level. The number of flashes of the LED per sequence will decrease by one.

▼ 10 To switch the device off, hold the button down for 3 to 4 seconds (click and hold) until the LED has stopped flashing.

▼ 11 If a geko™ device is to be applied to the other leg, repeat the steps above. Note that the LED and head of the device must be fitted to the outside of knee and the tongue to the back.

geko™

the device should be disposed of as other electrodes and dressings in accordance with local procedures in place at your hospital/clinic.

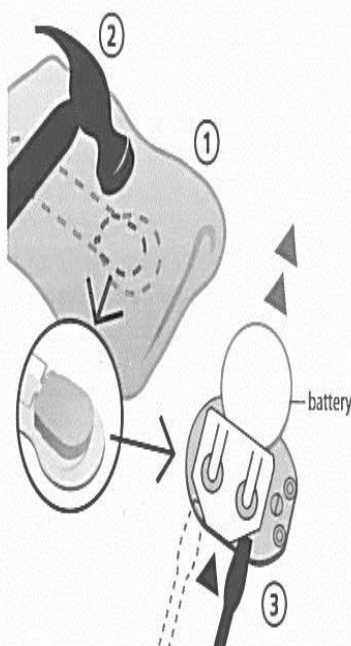


fig. 4 battery removal

5.0 Help

If you require any help with the use of the geko™ device, call: +44 (0)20 8238 6770

firstkind

living science

Firstkind Limited*

Daresbury Science and Innovation Campus,
Keckwick Lane, Daresbury, Cheshire WA4 4FS,
United Kingdom

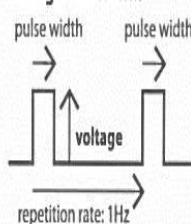
T: +44 (0)1925 607196 www.firstkind.co.uk

To find out more about geko™ visit:
www.geko.me

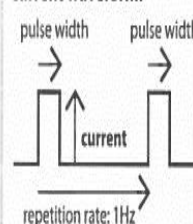
specification

product name	geko™
model reference	T-1
product type	powered muscle stimulator
class	BF
dimensions	149mm x 42mm x 11mm
weight	18g (geko™ device only)
power source	internally powered equipment, battery not replaceable
battery	primary lithium coin cell – removable for disposal
operation	continuous operation – equipment not suitable for use in presence of flammable anaesthetic mixture with air or with oxygen or nitrous oxide
stimulation modes	7 (selectable pulse widths)
pulse current	27mA ($\pm 15\%$) constant current, compliance to 80V
load impedance	200Ω to 3kΩ for 27mA output
pulse voltage	set by current and load
pulses width (200 Ω to 10k Ω)	70, 100, 140, 200, 280, 400 and 560μs ($\pm 5\%$ +20 μ s) (open circuit $\pm 5\%$ +70 μ s)
repetition rate	1Hz ($\pm 5\%$)
maximum charge	20μC per pulse
net charge output	zero per cycle
output coupling	ceramic capacitor

voltage waveform:



current waveform:



output voltages and currents:

measured at internal outputs of the pulse generator ($\pm 15\%$)

pulse width	half-power setting 280 μ s		full-power setting 560 μ s	
load	current	voltage	current	voltage
200 Ω	27mA	5.4V	27mA	5.4V

specification cont.

fault indication	the stimulator device will automatically switch off for over current, under current, low battery voltage or end of 30 hour run time
standards	IEC60601-1 (1996), IEC60601-2-10 (2001), IEC60601-1-2 (2001), ISO 10993

Operating conditions:

temperature range	10°C to 30°C
humidity range	10% to 80% non-condensing

Storage conditions in original packaging:

temperature range	-10°C to 30°C
humidity range	10% to 80% non-condensing
shelf-life	6 months when stored as above

Transport conditions:

temperature range	-10°C to 50°C
humidity range	10% to 80% non-condensing

materials	soft moulding: TPE Hydrogel cover: PET (Mylar) polyethylene terephthalate
warranty	6 months storage before use and 24 hours stimulation

symbols definition of symbols used on packaging

	type BF applied part, suitable for direct electrical contact to patient but not for direct cardiac application
	single use only geko™ devices should only be used once
	product contains no latex
	refer to instructions
	traceability number
	expiry date

Appendix 26 – Huntleigh Flowtron™ Instruction Manual

FLOWTRON®**DVT Prophylaxis Systems****Calf Garment DVT10/E****Standard - up to 43cm calf circumference****EN** *To be used only under the direction of a physician • Non-Sterile • 100% Latex Free • For Single Patient Use Only**For use with Huntleigh Healthcare Flowtron® Universal, Flowtron Excel and Flowtron DVT pumps only.***Instructions for Use**

1. Plug the pump into a suitable electrical outlet. Do not turn the pump on at this time.
2. If using the **Flowtron Excel** or **Flowtron DVT** systems, connect the L550 orange tubing set to the pump, ensuring a 'click' is heard from the snap-lock connectors.
3. Remove the garments from the sealed bag. The garments may be used on either leg. Unfold the garment and position the inflatable bladder directly behind the patient's calf.
4. Snugly wrap the garment around the patient's leg and secure the fastener tabs. Repeat for the other leg.
5. Attach the garments to the pump tubing set ensuring a 'click' is heard from each snap-lock connector.
6. If using **Flowtron Excel** or **Flowtron DVT** systems, adjust the pump pressure regulator dial to the recommended pressure unless otherwise ordered by the physician.
7. Turn the pump on. The green power indicator lights should illuminate.
8. Refer to the pump operating manual for complete information on the use of the system.

Indications

Intermittent Pneumatic Compression (IPC) is indicated for use for the prevention of deep vein thrombosis due to the presence of risk factors for thrombus formation during orthopaedic, trauma, urologic and general surgery. It is also suitable for other patient groups including neurology, critical care, general medical and obstetrics.

Contraindications

IPC should not be used in the following conditions:

1. Severe arteriosclerosis or other ischaemic vascular diseases.
2. Known or suspected acute Deep Vein Thrombosis (DVT) or phlebitis.
3. Severe congestive cardiac failure or any condition where an increase of fluid to the heart may be detrimental.
4. Pulmonary embolism.
5. Any local condition in which the garments would interfere, including gangrene, recent skin graft, dermatitis or untreated, infected leg wounds.

If you are unsure of any contraindications refer to the patient's physician before using the device.

Cautions

1. Garments should be removed immediately if the patient experiences tingling, numbness or pain.
2. When used for DVT prophylaxis, continuous use is recommended and any interruption of therapy for a substantial length of time should be at the discretion of the physician.

Symbols

Single Patient Use



Patient Name



Therapy Start Date

Huntleigh Healthcare Limited, 310-312 Dallow Road, Luton, Bedfordshire,
LU1 1TD, Tel +44 (0)1582 413104, Fax +44 (0)1582 459100

Appendix 27 – Kendall SCD Express™ Instruction Manual

SCD EXPRESS*

Section I - General Operating Instructions

Set up

- Place the Controller on the footboard via the bed hook or place it on a horizontal surface appropriate for the environment, such as on a table, within reasonable proximity to the point of use. Be sure to allow adequate air flow to the vents located on either end of the controller.
- The controller can operate with one or two garments attached to the patient.
- Plug the tubing set(s) into the back of the controller. Route the tubing toward the patient's limbs, being careful to leave access ways clear and eliminate tripping hazards.
- Plug the tubes into garment(s) wrapped onto the patient's limbs.
- Match the left and right ports with the left and right limbs of the patient. Although the operation of the controller is not affected, troubleshooting can be easier. Check tubing set(s) for kinking and secure attachment at the controller and the garment(s).
- Plug the controller power cord into a properly grounded hospital grade receptacle.
- Be certain that no flammable anesthetic gases are present.

Start-up

- Press the Power/Standby button to begin normal operation. No further user intervention is required unless there is a fault condition detected or if therapy must be discontinued.
- The Controller will beep and then flash all the LED's.
LED Indicator Sequence:

1. Port A Status, Port B Status, & Vascular Refill	→ GREEN
2. Port A Status, Port B Status, & Service Required Error	→ RED
3. Battery Status/Test Mode 1-3 & Test Mode/Error Code 4-8	→ GREEN
4. Battery Status/Test Mode 1-3 & Test Mode/Error Code 4-8	→ RED
- An automatic valve and pump test will be quickly performed as the system tests microprocessor function and system memory.
- The pump will begin to operate as part of the Automatic Garment Detection procedure.
- Detection of inoperative LED's and the audible alarm function at start-up is the user's responsibility.

Automatic Garment Detection

- Automatic Garment Detection will determine if a disposable garment has been properly connected to the controller.
- The controller will automatically detect the number and type(s) of garments connected [Leg Sleeve(s) or Foot Cuff(s)].
- If the controller senses an attached garment, then the corresponding Port Status Indicator (A or B side) on the front display will be lit green.
- If no garments are detected, the system will alarm until it is turned off. Check the garment application and tubing connections then restart Controller if necessary.

Note: If a garment is attached anytime after the Automatic Garment Detection Procedure has started, the system must be restarted to ensure that the proper therapy will be applied to the limb(s).

Normal Operation

- Verify that the corresponding Port Status Indicators are green for each disposable garment attached to the controller.
- After garments have been successfully detected, the Controller begins the process of applying intermittent compression alternating from one port to the other if two garments are attached. Otherwise, the controller applies compression to only one port when one garment is attached.
- On successive cycles, the controller automatically adjusts its operating parameters to maintain set pressure.

Pressure Settings

- The Controller features microprocessor-controlled automatic pressure adjustment.
- The pressure setting depends on the type of garment: 45 mmHg for Leg Sleeves; 130 mmHg for Foot Cuffs.

Appendix 28 – Discomfort Questionnaire for geko™ vs. IPC Comparison Study

REC Number: 05 / Q0604 / 11

Volunteer Number:

Time: 30 minutes

Date:

Device:

Discomfort Questionnaire

When compared to a blood pressure cuff inflated around your upper arm, how does the stimulation feel?

Q: “How uncomfortable was the last session with the device?”

Select one answer by circling the number.

① ② ③ ④ ⑤

[1] No sensation (other than muscles tensing and relaxing)

[2] Minimal sensations

[3] Mild discomfort

[4] Moderate discomfort

[5] Severe discomfort

Fold Here ----- **Fold Here**

Q: “What did the last session with the device feel like?”

Make a vertical line across the line below, which relates to the intensity of any discomfort experienced by the procedure.

No Sensations

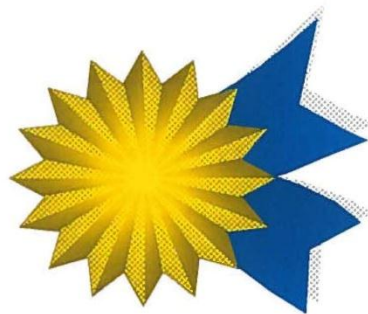
Severe Discomfort

Certificate of Completion

is hereby granted to:

Huda Jawad

to certify that they have completed to satisfaction



Research Governance Framework

Granted: 3rd June 2009



Yolanda Moyo/Helen Cadiou
Research Governance & GCP Manager